*Br. J. Pharmac.* (1984), **82**, 809–815

# Role of metabolism in the immediate effects and pneumotoxicity of 3-methylindole in goats

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**1** Rapid infusion of 3-methylindole (3-MI) dissolved in 10% Cremophor EL in water was immediately followed by pulmonary arterial hypertension, systemic arterial hypotension, decreased minute volume and periods of apnoea in goats.

**2** Rapid intravenous infusion of Cremophor EL/water alone caused similar immediate effects to those of Cremophor EL plus 3-MI in various dosages.

**3** Pretreatment of goats with piperonyl butoxide or phenobarbitone did not significantly alter these immediate cardiopulmonary responses. But pretreatment with piperonyl butoxide prevented clinical signs and pulmonary lesions of 3-MI toxicity, whereas phenobarbitone pretreatment shortened survival time and enhanced pulmonary pathology.

**4** Cremophor EL and 3-MI dissolved in Cremophor EL caused severe *in vitro* haemolysis of caprine and bovine erythrocytes.

**5** There was no relationship between the immediate effects of 3-MI and the subsequent development of 3-MI-induced pneumotoxicity and deaths in control goats or in goats pretreated with piperonyl butoxide or phenobarbitone. Induction or inhibition of mixed function oxidase activity had no influence on immediate responses to 3-MI but did change the severity of clinical and pathological responses.

**6** It is concluded that there is no apparent relationship between the immediate and the pneumotoxic effects of 3-MI. It is possible that the immediate effects are the result of intravascular haemolysis.

## Introduction

Fog fever is an acute respiratory distress syndrome which occurs in adult beef-type cattle soon after a change to better pasture in the autumn (Selman *et al.*, 1974). The main pulmonary lesions in fog fever are oedema, hyaline membranes, hyperplasia of alveolar type 2 pneumonocytes and interstitial emphysema (Pirie *et al.*, 1974). An identical condition, known under many synonyms of which acute bovine pulmonary oedema and emphysema (ABPE) is the most common, has been reported under similar circumstances on many occasions in the U.S.A., Canada and other countries (Maki, 1963). The available evidence supports the view that fog fever/ABPE is initiated by production of 3-methylindole (3-MI) from L-tryptophan in the rumen (Breeze & Carlson, 1982; Carlson & Breeze, 1983). This is a two-step process whereby L-tryptophan is first metabolized by ruminal microorganisms to form indole 3-acetic acid: indole 3-acetic acid is then decarboxylated by an anaerobic *Lactobacillus* species to produce 3-MI. The 3-MI is absorbed into the bloodstream from the rumen and metabolized by a mixed function oxidase (MFO) system, probably in lung, to cause pneumotoxicity. It appears unlikely that 3-MI is directly responsible for the pneumotoxicity seen in fog fever/ABPE since this compound is rapidly metabolized, is present in low concentrations in the target organ, and toxicity is influenced by pretreatment with MFO inducers and inhibitors (Carlson & Bray, 1983). Recent work indicates that a reactive intermediate(s) of 3-MI is formed by MFO metabolism and that cytotoxicity results from covalent binding of this intermediate to tissue macromolecules in specific lung cell populations (Carlson & Bray, 1983; Carlson & Breeze, 1983).

The various hypotheses on the aetiology of fog fever/ABPE which have been advanced over the years have fallen into two groups: (1) those that favour ingestion of a dietary toxin, and (2) those that suggest a hypersensitivity reaction in the lung

(Breeze & Carlson, 1982). Some common ground between both sets of ideas has been noted in the reports of increased plasma and ruminal 5-hydroxytryptamine (5-HT) levels in calves given L-tryptophan (Eyre, 1972) and in comparisons of the vasoconstrictive effects of 5HT, tryptamine and 3-MI *in vitro* (Eyre, 1975). Eyre (1980) has stated that 3-MI exerts pneumotoxicity by an anaphylactoid reaction, a view given some credence by the observation that rapid intravenous infusion of 3-MI in calves caused many of the immediate physiological responses reported in experimental bovine systemic anaphylaxis, including decreased systemic arterial pressure, increased pulmonary arterial pressure, decreased depth of respiration and periods of apnoea (Atkinson *et al.*, 1977). In view of the increasing body of evidence supporting a metabolic basis for 3-MI pneumotoxicity, we decided to clarify the relationship between the immediate responses to rapid 3-MI infusion and the pneumotoxic effects attributable to MFO metabolism.

## Methods

### *Animals*

Fifteen adult goats of mixed breeding and either sex were placed in individual pens for 1 to 2 weeks before the experiment and fed a diet of baled alfalfa hay with salt and water available *ad libitum*. Food was withheld for 12 h before anaesthesia. After determining body weight (b.wt), anaesthesia was induced with xylazine (0.22 mg $kg^{-1}$ b.wt) (Haver-Lockhart) and ketamine (11 mg $kg^{-1}$ b.wt) (Parke Davis) intramuscularly. Following induction, all goats were intubated and maintained on halothane (Ayerst) at a surgical plane of anaesthesia for the duration of the acute experimental period.

### *Intravenous infusions*

All infusions were given via an indwelling catheter in the left lateral saphenous vein. Cremophor EL (Sigma Chemical) was chosen as the solvent because it dissolves 3-MI well, is miscible with water and plasma, and had been used by Atkinson *et al.* (1977). In all experiments 3-MI (ICN Pharmaceuticals) was given as a 30 mg $ml^{-1}$ solution in 10% Cremophor EL in distilled water. The dosage procedure was standardized to the following sequence: (1) 10% Cremophor EL (volume equivalent to that of 16 mg 3-MI $kg^{-1}$ b.wt); (2) 1 mg 3-MI $kg^{-1}$ b.wt; (3) repeat 1 mg 3-MI $kg^{-1}$ b.wt; (4) 4 mg 3-MI $kg^{-1}$ b.wt; (5) 16 mg 3-MI $kg^{-1}$ b.wt, and (6) 28 mg 3-MI $kg^{-1}$ b.wt. Intravenous injections of 3-MI were infused at a standard rate of 5 mg $s^{-1}$, beginning 5 min after measured parameters had stabilized at a new baseline following the previous infusion. The total 3-MI dose was 50 mg $kg^{-1}$ b.wt.

Group (1) control goats, consisted of 3 males and 2 females. These goats were not pretreated with any drug. Group (2), 2 males and 3 females, was pretreated with 0.08 g phenobarbitone $kg^{-1}$ b.wt given orally, divided into two daily doses, at 72, 60, 48, 36, 24 and 12 h before 3-MI dosing. Group (3), 2 males and 3 females, was pretreated with 0.5 g piperonyl butoxide $kg^{-1}$ b.wt given intramuscularly at 24, 12 and 6 h before 3-MI dosing.

### *Instrumentation*

A 7 French Baltherm flow-directed balloon tipped catheter (Columbus Instruments) was directed into the pulmonary artery under fluoroscopy. A 6 French Swan-Ganz monitoring catheter (Edwards Laboratories) was inserted into the left carotid artery in the midcervical region and threaded 10 cm towards the base of the heart. Anatomical positioning of all catheters was verified via characteristic pressure tracings. The catheters were attached to Statham P 23 Db pressure tranducers (Gould) zeroed to the level of the scapulo-humeral joint with the animal in sternal recumbency. All catheters were continuously flushed with heparinized saline via a continuous flush system (Sorenson).

Minute and tidal volumes were recorded from an endotracheal tube attached to a Fleisch pneumotachograph (Dynasciences) connected to a Statham PM5ET (Gould) differential pressure transducer. All recordings were made on a Beckman R612 8 channel pen recorder. Intravascular pressures were electronically damped and recorded as mean pressures. Data were collected at the point of maximum increase or decrease for a given parameter within a 5 min period following the beginning of an injection.

Following completion of the last infusion, catheters were removed and all goats were given 12 mg oxytetracycline LA 200 (Pfizer) $kg^{-1}$ b.wt intramuscularly. Clinical signs of lung disease were monitored for 96 h or until death.

### *Post-mortem and histopathological methods*

All animals which survived to 96 h post-dosing were killed by injection of sodium pentobarbitone and immediately exsanguinated. All goats were examined post mortem. The lungs were removed, freed of the heart and mediastinal tissues and weighed. Sections of lung, liver, kidney, and any other organ showing gross lesions were fixed by immersion in 10% neutral buffered formalin. The middle lung lobe was fixed with 10% formalin by airway perfusion at 30 $cmH_2O$ pressure for 48 h, when 5 to 10 tissue

blocks were taken for processing. Tissues were embedded in paraffin, sectioned at 5 to 6 μm, and stained with haematoxylin and eosin for microscopic examination. A score of 0, 1, 2, 3 or 4 was given when up to 0, 25, 50, 75 or 100% of alveoli had oedema or alveolar epithelial hyperplasia. Five sections were graded for each lung.

In vitro *haemolysis*

This trial was undertaken to determine if Cremophor EL alone or with 3-MI had the ability to cause haemolysis. Whole blood was collected in heparinized vials from a young male goat and a young female calf. A solution of 10% Cremophor EL in distilled water was diluted with 0.9% w/v NaCl solution (saline) to yield final Cremophor EL concentrations of 10, 7.5, 5, 2 and 1%. An aliquot of each dilution was mixed with an equal volume of fresh whole heparinized blood. The mixtures were incubated for 5 min at room temperature then centrifuged for 15 min at 500 *g*. Supernatant absorption at 540 nm was determined using a Perkin-Elmer double beam spectrophotometer. This procedure was repeated with similar dilutions of 30 mg 3-MI $ml^{-1}$ 10% Cremophor EL.

*Statistical analysis*

Differences in cardiopulmonary parameters and lung scores were evaluated by randomization analysis according to the method of Siegel (1956) for a two sided test.

## Results

Baseline and experimental data for all goats are presented in Table 1. Before dosing, there was considerable variation in minute volume and systemic arterial pressure between individual goats in all groups. Mean values for pulmonary and systemic arterial pressures and for minute volume were not significantly different between groups, indicating that pretreatment with phenobarbitone or piperonyl butoxide did not have significant effects on cardiopulmonary function.

Dramatic increases in pulmonary arterial pressure (PAP) were noted in all groups immediately after injection of the Cremophor/water vehicle alone (Table 1). Systemic arterial pressure ($P_A$) fell in the control group (1) and rose slightly in groups (2) and (3). Minute volume ($V_1$) decreased in all groups and periods of apnoea were observed in some goats from all groups. There were no significant differences between groups in the magnitude of the PAP, $V_1$ and apnoeic responses.

After the first injection of 1 mg 3-MI $kg^{-1}$, PAP increased and $P_A$ and $V_1$ both decreased in all groups. Periods of apnoea were noted in goats of groups (2) and (3) (Table 1).

With successive doses of 3-MI (Table 1), PAP

**Table 1** Cardiopulmonary responses of goats to injection of Cremophor EL and water vehicle and sequential doses of 3-methylindole (3-MI)

| *Group No* | *Baseline parameter value* | *Treatment and % increase (↑) or decrease (↓) in mean measured parameter over baseline* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Cremophor EL* | 3-MI 1 mg $kg^{-1}$ | 3-MI 1 mg $kg^{-1}$ | 3-MI 4 mg $kg^{-1}$ | 3-MI 16 mg $kg^{-1}$ | 3-MI 28 mg $kg^{-1}$ |
| (1) | PAP – 28 ± 1.3 | ↑40 ± 10.2$^A$ | ↑23 ± 9.4$^A$ | ↑11 ± 5.4$^A$ | ↑15 ± 6.7$^A$ | ↑ 6 ± 7.6$^A$ | ↑15 ± 14.8$^A$ |
| | $P_A$ – 113 ± 14.8 | ↓ 7 ± 12.1$^A$ | ↓13 ± 9.4$^A$ | ↓14 ± 8.9$^A$ | ↓17 ± 7.6$^A$ | ↓42 ± 8.1$^C$ | ↓48 ± 7.6$^C$ |
| | $V_1$ – 12 ± 2.7 | ↓30 ± 6.7$^A$ | ↓28 ± 7.2$^A$ | ↓41 ± 5.4$^A$ | ↓38 ± 9.4$^A$ | ↓62 ± 5.4$^B$ | ↓ 6 ± 38.5$^B$ |
| | Ap – 0 | 4 ± 3.6 | 0 | 0 | 4 ± 2.7 | 116 ± 75 | 208 ± 88.6 |
| (2) | PAP – 26 ± 2.7 | ↑37 ± 12.1$^A$ | ↑ 9 ± 2.2$^B$ | ↑ 7 ± 3.6$^B$ | ↑ 2 ± 4.0$^B$ | ↑ 1 ± 5.5$^B$ | ↑ 8 ± 2.2$^B$ |
| | $P_A$ – 102 ± 8.9 | ↓ 8 ± 8.5$^A$ | ↓ 4 ± 3.1$^A$ | ↓ 5 ± 0.9$^A$ | ↓17 ± 2.2$^B$ | ↓37 ± 4.4$^C$ | ↓42 ± 3.6$^C$ |
| | $V_1$ – 7 ± 1.8 | ↓31 ± 8.5$^A$ | ↓12 ± 16.5$^A$ | ↓27 ± 6.2$^A$ | ↓35 ± 11$^A$ | ↓65 ± 5.5$^B$ | ↓46 ± 12$^B$ |
| | Ap – 0 | 3 ± 2.2 | 2 ± 1.3 | 1 ± 1.3 | 3 ± 1.8 | 79 ± 35 | 11 ± 72 |
| (3) | PAP – 26 ± 1.3 | ↑47 ± 12$^A$ | ↑36 ± 6.2$^C$ | ↑16 ± 3.1$^B$ | ↑31 ± 6.7$^B$ | ↑19 ± 8.5$^B$ | ↑ 4 ± 2.2$^B$ |
| | $P_A$ – 113 ± 13.4 | ↓ 5 ± 6.7$^A$ | ↓ 9 ± 2.2$^A$ | ↓ 9 ± 1.3$^A$ | ↓24 ± 4.0$^B$ | ↓37 ± 3.1$^C$ | ↓37 ± 4.9$^C$ |
| | $V_1$ – 17 ± 3.6 | ↓14 ± 9.4$^A$ | ↓29 ± 8.1$^A$ | ↓ ± 6$^C$ | ↓51 ± 9.8$^A$ | ↓47 ± 15.2$^B$ | ↓40 ± 9.4$^B$ |
| | Ap – 0 | 10 ± 6.7 | 4 ± 2.2 | 2 ± 2.2 | 40 ± 26.8 | 95 ± 47.4 | 30 ± 16.1 |

Values shown represent % increase or decrease over baseline before each injection. Values given ± s.e.mean.
PAP – pulmonary arterial pressure (mmHg); $P_A$ – systemic (carotid) arterial pressure (mmHg); $V_1$ – minute volume (L); Ap – duration of apnoea (s). Group (1) goats were given no pretreatment, Group (2) were given phenobartitone and Group (3) were given piperonyl butoxide. For each parameter, values with different superscripts are significantly different ($P < 0.05$).

**Table 2** Disposition, clinical responses and pathological findings in goats given 3-methylindole after no pretreatment (Group 1), pretreatment with phenobarbitone (Group 2) and pretreatment with piperonyl butoxide (Group 3).

| *Group* | *Goat number* | *Time to death after dosing (h)* | *Lung weight (% body weight)* | *Clinical response* | *Lung score* |
|---|---|---|---|---|---|
| 1 | 1 | 96 | 1.1 | Increased | 1 |
| No | 2 | 94 | 2.7 | rate and | 2 |
| pretreatment | 3 | 48 | 4.1 | depth of | 3 |
| | 4 | 72 | 1.9 | breathing: | 2 |
| | 5 | 96 | 1.9 | laboured respiration terminally in goats 2, 3, & 4 | 1 |
| mean ± s.e.mean | | 81.2 ± 9.5$^{A}$ | 2.3 ± 0.5$^{A}$ | | 1.9 ± 0.4$^{A}$ |
| 2 | 6 | 12 | 3.8 | Increased | 3 |
| Phenobarbitone | 7 | 34 | 1.5 | rate and | 1 |
| pretreatment | 8 | 47 | 2.0 | depth of | 2 |
| | 9 | 62 | 2.8 | breathing, | 3 |
| | 10 | 39 | 1.9 | laboured respiration terminally in all goats | 3 |
| mean ± s.e.mean | 38.3 ± 8.2$^{B}$ | 2.4 ± 0.4$^{A}$ | | 2.4 ± 0.4$^{A}$ | |
| 3 | 11 | 96 | 0.9 | No clinical | 0 |
| Piperonyl | 12 | 96 | 0.85 | abnormalities | 0 |
| butoxide | 13 | 96 | 0.9 | detected | 0 |
| pretreatment | 14 | 96 | 0.95 | | 0 |
| | 15 | 96 | 2.5 | Dull, fever, cough, increased respiratory rate | 0 |
| mean ± s.e.mean | | 96 ± 8.2$^{C}$ | 1.5 ± 0.1$^{B}$ | | 0$^{B}$ |

For each parameter, values with different superscripts are significantly different ($P < 0.05$).

generally increased, with the exception of group (2) after 16 mg and 28 mg 3-MI $kg^{-1}$, $P_A$ fell, $V_1$ decreased and the duration of apnoea became prolonged in all groups. These responses occurred after every 3-MI injection but did not appear to be 3-MI dose-dependent. Several goats in all groups required artificial resuscitation after the 16 and 28 mg $kg^{-1}$ doses.

Clinical signs, survival time and lung scores are shown in Table 2. Goats in group (1) had increased rate and depth of breathing, goats in group (2) were in respiratory distress and those in group (3) had no clinical signs of 3-MI toxicity. Lung lesions were more severe in group (2) than group (1); group (3) had no significant 3-MI lesions. The varied immediate responses after dosing, both within a group

**Table 3** *In vitro* haemolysis by dilutions of Cremophor EL in saline measured by supernatant absorption at 540 nm

| *Final Cremophor EL concentration* | 0% (Saline) | 1% | 2% | 5% | 7.5% | 10% |
|---|---|---|---|---|---|---|
| *Goat blood* | | | | | | |
| Absorption units | 1.5 | 1.5 | 1.5 | 23.5 | 31.5 | 38.5 |
| % increase | 0 | 0 | 0 | 1,567 | 2,100 | 3,567 |
| *Calf blood* | | | | | | |
| Absorption units | 4.0 | 4.0 | 4.0 | 4.0 | 17.5 | 40.5 |
| % increase | 0 | 0 | 0 | 0 | 438 | 1,013 |

**Table 4** *In vitro* haemolysis by dilutions in saline of 30 mg 3-methylindole $ml^{-1}$ 10% Cremophor EL measured by supernatant absorption at 540 nm

| *Dilution of Cremophor EL/3-MI solution* | Control (saline) | 1:10 | 1:5 | 1:2 | 1:1.3 | 1:0 |
|---|---|---|---|---|---|---|
| *Goat blood* | | | | | | |
| Absorption units | 1.5 | 21.0 | 24.0 | 21.0 | 34.0 | 31.5 |
| % increase | 0 | 1400 | 1600 | 1400 | 2267 | 2100 |
| *Calf blood* | | | | | | |
| Absorption units | 3.5 | 23.5 | 19.0 | 25.5 | 22.0 | 21.0 |
| %increase | 0 | 671 | 543 | 729 | 629 | 600 |

and between groups, shown in Table 1, had no predictive value in relation to subsequent clinical signs or pathology (Table 2).

Cremophor EL and 3-MI dissolved in Cremophor EL caused significant *in vitro* haemolysis (Tables 3, 4). Calf erythrocytes appeared to be more resistant to haemolysis by these solutions than did caprine erythrocytes.

## Discussion

The results of this experiment indicate that rapid intravenous infusion of Cremophor EL/water vehicle and 3-MI in Cremophor/water cause significant immediate physiological changes in the cardiopulmonary systems of anaesthetized goats comparable to those reported in calves by Atkinson *et al.* (1977). Cremophor alone caused greater increases in pulmonary arterial pressure in all treatment groups than did cremophor plus 3-MI in the various dosages. This was not true for other recorded parameters. Increasing dosages of 3-MI in Cremophor caused progressively greater depression of systemic arterial pressure and minute volume and prolongation of apnoea. There were no significant differences between groups in responses to Cremophor alone, except for systemic arterial pressure, which fell in the control group (1) and rose in groups (2) and (3). After Cremophor and 3-MI, differences between groups were usually the result of the phenobarbitone pretreatment group (2) showing a lesser reaction than the other two groups.

Pretreatment with piperonyl butoxide prevented clinical signs and lesions of 3-MI pneumotoxicity in the goats of group (3), where as phenobarbitone enhanced these responses, as noted by Bray & Carlson (1979). But the immediate physiological responses seen after rapid infusion of 3-MI were generally similar in all groups and, if anything, tended to be less in goats pretreated with phenobarbitone. There was no relationship between the immediate effects of 3-MI administration described here and by Atkinson *et al.* (1977) and the subsequent development of 3-MI-induced pneumotoxicity and deaths, and so it appears that the two events are unrelated. Induction or inhibition of MFO activity has no influence on the immediate responses to 3-MI but does change the severity of clinical and pathological responses.

Atkinson *et al.* (1977) administered 3-MI in Cremophor EL to calves. Findings were similar to those described here except that a dramatic difference in the degree of response was noted between the first administration of 1 mg 3-MI $kg^{-1}$ and a repeat of the same dose. The first dose was followed by severe cardiopulmonary alterations while the second dose produced either no response or a very mild reaction. Atkinson *et al.* (1977) speculated that the immediate reaction to 3-MI was an anaphylactoid response and that development of tachyphylaxis was responsible for the disparity between the first and second doses. This phenomenon was observed only to a very slight degree in the goats used in this trial. Atkinson *et al.* (1977) also found that the immediate physiological effects of 3-MI in calves were abolished by predosing with sodium meclofenamate, a known inhibitor of bovine anaphylaxis (Eyre *et al.*, 1973). Whatever the influence of sodium meclofenamate on the immediate effects of 3-MI, this drug has been shown to have no benefit in the protection of cattle against the clinical and pathological syndrome of 3-MI-induced ABPE (Breeze, 1978).

Numerous physiological trials have been performed on calves during experimental anaphylaxis (Aitken & Sanford, 1969; Wells *et al.*, 1973; Eyre *et al.*, 1973). Findings generally consist of a biphasic increase in pulmonary arterial pressure, decreased heart rate, systemic arterial pressure, and minute volume and prolonged periods of apnoea. Animals characteristically respond only to the first dose of antigen and are refractory thereafter. Following the immediate reaction, animals either die of cardiovascular collapse, pulmonary oedema and anoxia or recover uneventfully. The delayed development of pulmonary lesions similar to those of ABPE in the 96 h subsequent to anaphylaxis has not been reported. The responses seen in the goats in this study

were different from those of anaphylaxis in that there was no biphasic rise in pulmonary arterial pressure, all parameters continued to show alterations with repeated dosing, and most parameters were dose-dependent.

Atkinson *et al.* (1977) used pentobarbitone to anaesthetize their calves. This did not prove suitable for the goats in this study because phenobarbitone and butoxide pretreatments respectively greatly diminish and greatly prolong the duration of anaesthesia. Halothane was chosen as the primary anaesthetic for maintenance of experimental animals. The advantages were a low degree of hepatic metabolism, allowing use in animals with modified hepatic MFO systems, and a degree of anaesthetic stability not achievable with parenteral anaesthetic agents. Halothane does cause myocardial and respiratory depression, but these effects stabilize or even return to normal within 30 min of induction of anaesthesia (Hall, 1957; Eberly *et al.*, 1968). Physiological data were not gathered for 90 min following induction of anaesthesia to circumvent artifactual data generated by the inducing agents or by halothane. Goats given identical doses of the xylazine/ketamine combination used in this trial were fully recovered at 40 to 45 min post injection (Kumar *et al.*, 1976), and so it is unlikely that the inducing agents had significant effects on measured responses.

Inert organic solvents used for drug administration frequently demonstrate a degree of cardiopulmonary effect. The polyoxyethylene derivatives of hexitol anhydride partial fatty esters, such as Tween 20, 40, 60, and 80, depress arterial blood pressure at low dosages in the monkey, cat, rat, chicken, guinea-pig, and rabbit, and cause death at high dosages. They produced severe hypotension and an 'anaphylactic' response in members of the genus *Canis* (Krantz *et al.*, 1948). Cremophor EL is a non-ionic polyoxyethylated emulsifier closely related to the above compounds and is known to have a similar effect in *Canidae* (Child *et al*, 1971). Cremophor EL causes systemic arterial hypertension in conscious cats followed by pruritis, flushing of the ears, and oedema of the paws, ears, and facial skin (Child *et al.*, 1972). Cremophor EL caused many of the same cardiopulmonary changes (varying only in degree) as those attributable to 3-MI in this experiment, which was an important difference from the study of Atkinson *et al.* (1977). Atkinson *et al.* (1977) found no cardiopulmonary changes in pentobarbitone-anaesthetized calves with Cremophor EL alone. Differences in species or anaesthetic response could provide an immediate explanation of this discrepancy. But Olcott (1981) found no significant differences in physiological responses to two sequential injections of Cremophor EL in a calf anaesthetized with halothane after xylazine/ketamine induction and the same calf anaesthetized with pentobarbitone. Under both anaesthetics, minute volume decreased and pulmonary arterial and systemic arterial pressures increased to the same degree immediately after each Cremophor injection and changes were of equal magnitude after the first and second dose. A conclusive explanation for the apparent lack of response to Cremophor noted by Atkinson *et al.* (1977) is not possible, but species and anaesthetic do not appear to account for the differences.

It appears unlikely that the response to Cremophor EL or to Cremophor EL and 3-MI in goats and calves is anaphylactic in nature due to the repeatability of the reaction, the dose-dependent response, and the unlikelihood of prior sensitization to an infrequently used solvent. Another explanation is provided by the observation that injection of autogenous haemolysed red cells causes dramatic depression of systemic arterial pressure and increased pulmonary arterial pressure in dogs (McBrady *et al.*, 1978). This finding may be of considerable significance since polyoxyethylene derivative solvents cause haemolysis *in vitro* and *in vivo* (Krantz *et al.*, 1948), as do propylene glycol (Gross *et al.*, 1979) and 3-MI (Bray & Carlson, 1975). We found that Cremophor EL and 3-MI dissolved in Cremophor EL causes severe *in vitro* haemolysis of caprine and bovine erythrocytes. At least some of the immediate effects of Cremophor EL and Cremophor EL plus 3-MI may, therefore, be the result of intravascular haemolysis caused by high local concentrations during rapid injection.

The purpose of this experiment was to demonstrate a relationship between the acute cardiopulmonary effects of 3-MI and the development of typical 3-MI-induced ABPE. Since there is no apparent relationship between the immediate and the pneumotoxic effects of 3-MI it must be assumed that the two events are unrelated. The data from this experiment do not allow a conclusive statement regarding the aetiology of the acute effects in goats but it would appear that the response is similar to that caused by a variety of lipophilic molecules and is not anaphylactic in origin.

Supported by ARC project 0399. Reprint requests to R.G.B., Washington State University Agricultural Experiment Station Scientific Paper Number 6723.

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(*Received November 4, 1983. Revised January 23, 1984.*)