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# EFFECTS OF 3-METHYLINDOLE IN CATTLE

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**1** Rapid intravenous injection of 3-methylindole (3-MI) was shown to induce an anaphylactoid-like reaction in calves.

**2** This was suggested by the reduction in response to a repeat dose of 3-MI, by the reduction of effects in the presence of antagonists to the putative mediators of anaphylaxis in cattle and by the production of signs similar to those seen in experimentally induced bovine anaphylaxis.

**3** The plasma half-life of 3-MI was short (14.4 min) and, since absorption of 3-MI from the rumen is known to be slow, the extent of formation of 3-MI from L-tryptophan in the rumen would have to be substantial if 3-MI is to be considered the causative agent of 'fog fever', an acute respiratory distress syndrome seen in cattle.

## 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, Wiseman, Breeze, Pirie & Bogan, 1976). The aetiology of fog fever is unknown but it is possible that the condition is the result of an anaphylactic reaction or is caused by the ingestion of a toxic substance in herbage. Most recent investigations have examined the role of pasture constituents, since it has been found that oral doses of the amino acid, L-tryptophan, can give rise to a proliferative alveolitis similar to that seen in fog fever (Dickinson, Spencer & Goreham, 1969). Intravenous doses of L-tryptophan do not induce similar lesions and it is probable that a ruminal metabolite of L-tryptophan is responsible for the lung lesions. The only such metabolite of L-tryptophan which is known to be toxic is 3-methylindole (3-MI; skatole), a compound formed by ruminal microorganisms via indole 3-acetic acid as an intermediate (Carlson, Yokoyama & Dickinson, 1972). A severe proliferative alveolitis occurs 24 to 96 h after oral dosage or slow (6 to 12 h) intravenous infusion of 3-MI (Carlson *et al.*, 1972; Pirie, Breeze, Selman & Wiseman, 1976).

The purpose of this study was to examine the acute effects when 3-MI was given *rapidly* to cattle and to assess whether 3-MI itself had direct pneumotoxic effects, since it was possible that some further metabolite of 3-MI could be involved. The plasma half-life of 3-MI was also measured since the kinetics of 3-MI, i.e. the rate and extent of formation in the rumen, the rate of absorption from the rumen, and the rate of plasma clearance, are important in determining the plasma concentration and thereby the likelihood of 3-MI being the cause of field outbreaks of fog fever.

## Methods

### *Animals*

Fourteen parasite-free calves between two and 12 months old were used; the animals were anaesthetized with pentobarbitone (20 mg/kg, i.v.) with additional 2 mg/kg doses intravenously given as required throughout the course of the experiments to maintain anaesthesia.

### *Intravenous infusions*

All previous intravenous administrations of 3-MI have been made using propylene glycol as a solvent (Carlson *et al.*, 1972; Pirie *et al.*, 1976). This solvent is unsuitable for rapid intravenous infusions because of its viscosity so all previous experiments have involved administration over a period longer than 6 hours. Initially we tried ethyl alcohol as a solvent but this was found to be unsatisfactory because 3-MI precipitated out on infusion and also because the alcohol caused venous thrombosis. The solvent chosen was 'Cremophor EL' (Victor Blagdon & Co.), a polyoxyethylated castor oil. This solvent dissolves 3-MI well, is miscible with water and plasma and 3-MI does not precipitate out on infusion. In all experiments 3-MI (Sigma London Chemical Co. Ltd) was administered

as a 30 mg/ml solution in 10% 'Cremophor EL' in distilled water.

Infusions were made into the femoral vein via an indwelling cannula and blood sampling for 3-MI determinations was from the other femoral vein. Throughout each experiment, a record was made of carotid arterial pressure, standard limb lead (lead II) ECG, the heart rate from an instantaneous heart rate meter, and respirations via a Fleisch pneumotachograph. Pulmonary arterial pressure was also monitored via an indwelling catheter introduced via the jugular vein; the position of the catheter was verified at necropsy. Arterial pressures were measured with Bell and Howell electronic transducers (Type 4-422-0001) adjusted to the elevation of the anaesthetized animal. All recordings were made on a Devices M19 8-channel pen recorder. Mean arterial pressure was calculated from the formula MAP = Pd + Ps − Pd/3 where Pd = mean diastolic pressure and Ps = mean systolic pressure.

In all experiments the dosage procedure was standardized with intravenous infusion of the dose taking place over 1 min; blood samples for 3-MI determination taken at 2, 5, 10, 20 and 30 min after the end of infusion; infusion of the next dose beginning at 30 minutes.

*Drugs*

Mepyramine maleate (May & Baker Ltd) (5.0 mg/kg, i.m.), cyproheptadine hydrochloride (Merck, Sharp & Dohme Ltd) (0.3 mg/kg, i.v.) and sodium meclofenamate (Parke, Davis & Co.) (2.0 and 10.0 mg/kg, i.v.) were administered 20 min, 5 min and 5 min respectively before 3-MI administration according to the methods of Aitken & Sanford (1972). Indomethacin was administered intravenously as a 20 mg/ml solution in 95% alcohol 20 min before 3-MI administration.

*Measurement of plasma concentrations of 3-methylindole*

In six calves (Nos. 3, 4, 5 and 6 (Table 2) and two others, Nos. 13 and 14 in which physiological measurements were not made) plasma levels of 3-MI were measured at times after 3-MI administration until 30 min after the 8 mg/kg dose. The estimation is based on that described by Bradley & Carlson (1974); 1 ml of plasma was shaken for 5 min with 10 ml re-distilled *n*-hexane. The plasma was pipetted off and the hexane layer was concentrated under nitrogen at 50°C to 1 ml and a 5 µl aliquot was injected into the gas chromatograph. 3-MI concentrations were quantified from known standards of 3-MI in *n*-hexane. The chromatographic estimations were made with a Pye 104 gas chromatograph with flame ionization detector. A glass column (1 m × 5 mm) packed with 10% DC 200 on Gas-Chrom Q (100–200 mesh) was used at a column temperature of 150°C and a detector temperature of 250°C. Under these conditions the retention time of 3-MI was about 3 minutes.

Post mortem *and histopathological methods*

Animals 1 and 3 died during the course of the experiment and calves 4 and 7 were killed with pentobarbitone shortly after recovery. These four calves were examined *post mortem* and representative portions of tissue were taken from the lungs, tracheobronchial system and other organs as required. Tissues were fixed in 10% formalin or corrosive formol, dehydrated and double embedded in celloidin and paraffin wax in a vacuum. All sections were cut at 6–8 µm and stained with haematoxylin and eosin.

## Results

*Response to 'Cremophor EL'*

The solvent used (10% 'Cremophor EL' in distilled water) was administered to four calves (Calf numbers 3, 4, 5, 6) during anaesthesia and before administration of 3-MI at a volume equivalent to that used to deliver the highest dose of 3-MI (i.e. 16 mg/kg). No effects were seen on any of the parameters measured.

*Response to 3-methylindole*

Since doses of 3-MI as high as 60 mg/kg have been infused intravenously over periods of 6–12 h (Carlson, Dickinson, Yokoyama & Bradley, 1975), it was surprising that an initial dose of 4 mg/kg proved fatal in the two calves used; these animals (Nos. 1 and 2) died within 2 min of the end of the injection. By reducing the initial dose it was found that 1 mg/kg was the highest dose that could be given without causing acute deaths.

The response of six calves (Nos. 3 to 8) to an initial 3-MI dose of 1 mg/kg is shown in Table 1. The response varied in each animal but the most consistent finding was pulmonary arterial systolic hypertension, beginning 1 to 3 min after the completion of the infusion, followed by a period of apnoea then a prolonged period of marked tachypnoea and hyperpnoea. Pulmonary arterial diastolic pressure also increased in two of the calves (Nos. 7 and 8) but to a lesser degree than the systolic pressure and in one calf (No. 6) decreased slightly. In all three, however, the mean pulmonary arterial pressure increased.

The marked fall in carotid arterial pressure was not accompanied or followed by a reflex tachycardia. In all cases no change or a slight fall in heart rate was observed with Figures 1 and 2 showing typical responses.

Repetition of the dose of 1 mg/kg 30 min later was

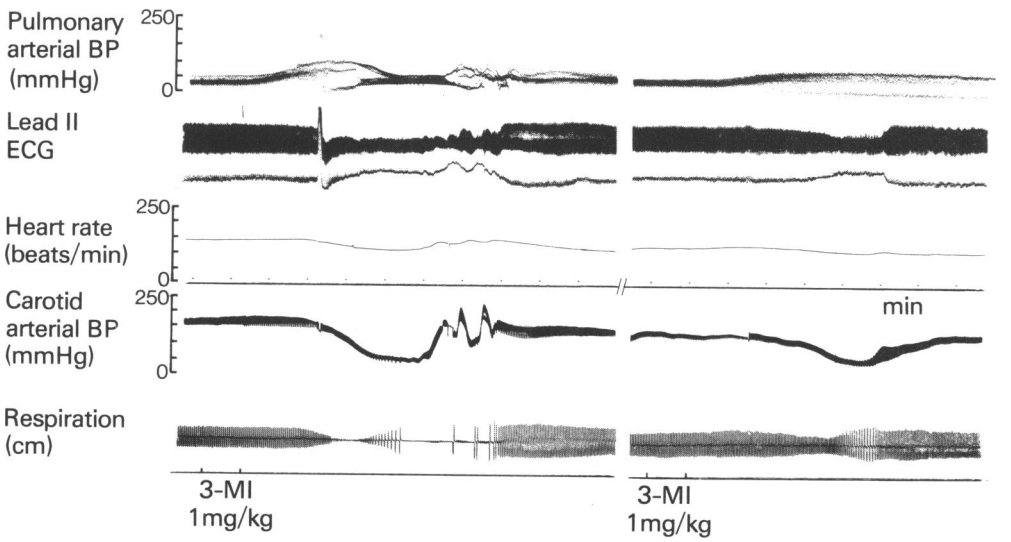


**Figure 1** Response of calf (No. 6) to 1 mg/kg 3-methylindole (3-MI) administered intravenously and repeated 30 min later.

accompanied by similar but much reduced responses in all six calves. Typical responses to the initial and repeat doses of 1 mg/kg are shown in Figure 1. Increasing the dose of 3-MI to 2, 4, 8 and 16 mg/kg resulted in similar but more immediate and dose-related effects on respiration which were also accompanied by pulmonary hypertension. Typical responses (Calf No. 4) are shown in Figure 2. The responses of the calves are shown in Table 2.

*Effects of antagonists on the response to 3-methylindole*

A mixture of antagonists, *viz.* mepyramine, cyproheptadine and indomethacin given to one calf completely eliminated the responses to 3-MI 1 mg/kg. This calf (No. 4) had reacted markedly to this dose of 3-MI one month previously (Table 1).

Two other calves (Nos. 9 and 10) pretreated with

**Table 1** Response of calves to an initial dose of 3-methylindole 1 mg/kg and repeated 30 min later

| *Calf. No.* | *3-Methylindole dose (1 mg/kg)* | *Respiratory depth max. decrease (%)* | *Mean carotid arterial blood pressure max. decrease (mmHg)* | *Mean pulmonary arterial blood pressure max. increase (mmHg)* |
|---|---|---|---|---|
| 3 | Initial | 40 | 30 | ND |
| | Repeat | 0 | 0 | |
| 4 | Initial | 80 | 90 | ND |
| | Repeat | 0 | 0 | |
| 5 | Initial | 100(30)* | 40 | ND |
| | Repeat | 50 | 0 | |
| 6 | Initial | 100(80)* | 110 | 20 |
| | Repeat | 20 | 70 | 0 |
| 7 | Initial | 0 | 0 | 55 |
| | Repeat | 0 | 0 | 15 |
| 8 | Initial | 100(40)* | 90 | 30 |
| | Repeat | 30 | 15 | 10 |

*= Duration of apnoea in seconds; ND = not determined.

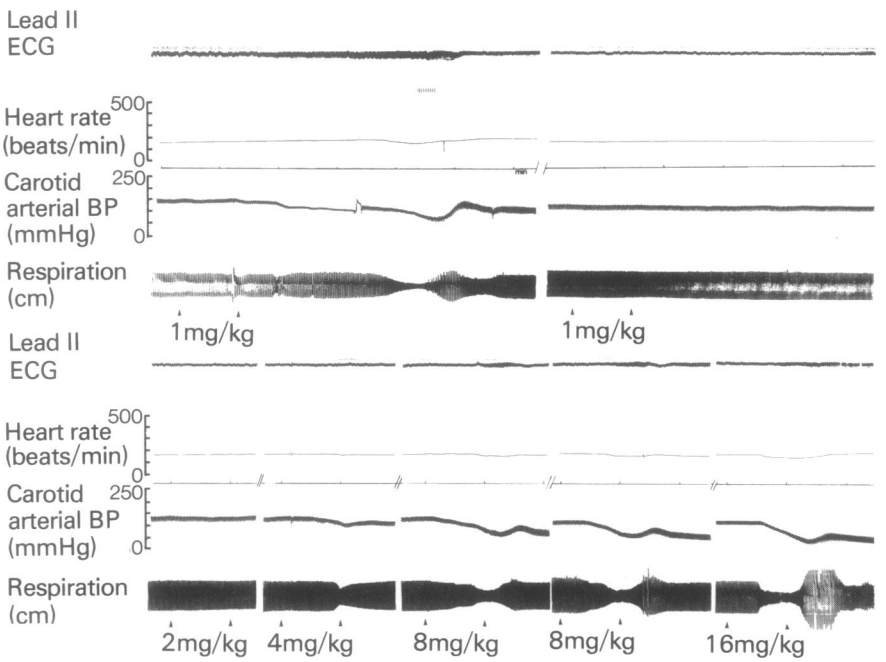


**Figure 2** Response of calf (No. 4) to successive doses (1, 1, 2, 4, 8, 8 and 16 mg/kg) of 3-methylindole administered intravenously at 30 min intervals.

**Table 2** Responses of calves to successive doses of 3-methylindole given at 30 min intervals

| *Calf No.* | *3-Methylindole dose (mg/kg) 30 min intervals* | *Response* |
|---|---|---|
| 1 | 4 | Died 1 min post injection |
| 2 | 4 | Died 2 min post injection |
| 3 | 1,1,2,4,8,16 | Died 30 min post 16 mg/kg |
| 4 | 1,1,2,4,8,8,16 | Survived. Respiratory rate elevated for 6 days |
| 4† | 1 | Survived. No respiratory changes on recovery |
| 5 | 1,1,2,4,8,16 | Died 2 min post 16 mg/kg |
| 6 | 1,1,2,4,8,16 | Survived. Respiratory rate elevated for 7 days |
| 7 | 1,1 | Survived. Respiratory rate elevated for 6 hours |
| 8 | 1,1 | Survived. Respiratory rate elevated for 12 hours |
| 9* | 1 | Survived. No respiratory changes on recovery |
| 10* | 1 | Survived. No respiratory changes on recovery |
| 11** | 1 | Survived. No respiratory changes on recovery |
| 12** | 1 | Survived. No respiratory changes on recovery |

* Pre-treated with sodium meclofenamate (2 mg/kg); ** pre-treated with sodium meclofenamate (10 mg/kg); † pre-treated with mepyramine, cyproheptadine and indomethacin.

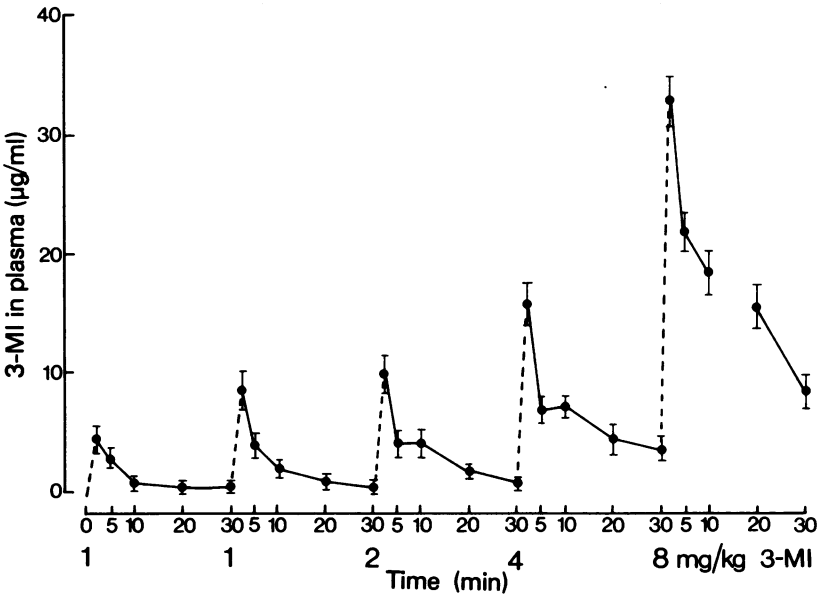


**Figure 3** Mean plasma concentrations of 3-methylindole (3-MI) in 6 calves after administration of successive doses (1, 1, 2, 4 and 8 mg/kg) of 3-methylindole intravenously at 30 min intervals. Vertical lines show s.e. means.

sodium meclofenamate (2.0 mg/kg, i.v.) alone showed only a slight reaction to an initial dose of 1 mg/kg 3-MI, respiratory depth being reduced by 30 and 10% respectively with no period of apnoea. In two other calves (Nos. 11 and 12) pretreated with a higher dose of sodium meclofenamate (10 mg/kg, i.v.) no effects on any of the parameters measured were seen at any time after 1 mg/kg 3-MI.

Post mortem *findings*

In calves 1 and 3 (Table 2), which died during the experimental procedure, there was severe pulmonary congestion and oedema. Lung lobules were dark red and fluid ran from their cut surfaces on pressure. Moderate interlobular oedema was also present and white frothy fluid filled the tracheobronchial system. Interstitial emphysema was noted in calf No. 1 and many lung lobules were overinflated in both calves. The congestion and oedema were apparent histologically. Respiratory acini were filled with oedema fluid. Capillaries of the inter-alveolar septa were intensely congested and neutrophils were frequently seen in these vessels. The lamina propria of bronchi and bronchioles was oedematous, particularly in calf No. 3. In this animal, the lung lesions were more severe and additional findings were intra-alveolar haemorrhage, a light exudate of neutrophils and macrophages in the lumina of many small bronchioles and the presence of infrequent globule leukocytes in the basal part of the tracheobronchial epithelium.

Calves 4 and 7 were destroyed by injection of pentobarbitone 8 h and 6 h respectively after the last injection of 3-MI: there were no residual clinical abnormalities at this stage. There was a moderate degree of pulmonary congestion and mild alveolar oedema in scattered acini in both cases. Neutrophils were frequently seen in the capillaries of the interalveolar septa. Animal 7 had lesions of cuffing pneumonia in the cranial lung lobes but the other 3 calves (Nos. 1, 3 and 4) did not have significant lesions of this type.

*Plasma half-life of 3-methylindole*

The mean plasma concentrations of 3-MI in six calves are shown in Figure 3. After each dose of 3-MI the plasma concentration decreased biphasically with a rapid decline within 5 min and a slower decline from 5 to 30 minutes. Since the initial rapid fall in 3-MI levels is probably a redistribution effect, the mean plasma half-life of 3-MI for doses of 3-MI between 1 and 8 mg/kg (Figure 3) between 5 and 30 min after administration was $14.4 \pm 2.5$ min (mean $\pm$ s.e. mean).

## Discussion

The evidence from this work for 3-MI to be considered the causative agent of fog fever in cattle is conflicting.

On the one hand the clinical and pathological signs seen after 3-MI administration are similar to those seen in field cases of fog fever. On the other hand, the kinetics of 3-MI, the slow rate of absorption from the rumen and rapid plasma half-life, would require that formation of 3-MI from L-tryptophan is rapid and extensive.

A small dose of 3-MI administered rapidly intravenously gives rise to an anaphylactoid-like reaction in cattle. This is suggested by (1) production of clinical signs similar to those seen during experimental anaphylaxis in calves, including pulmonary hypertension, systemic hypotension, dyspnoea and apnoea (Aitken & Sanford, 1969); (2) reduced response on repeating the initial dose, presumably due to depletion of mediators, and (3) reduction or abolition of the initial effects by antagonists to known mediators of anaphylaxis in cattle (Eyre, 1971). This reaction may be the result of 3-MI acting as a hapten in an immune reaction or, more directly, by a non-specific mechanism causing release of mediators of anaphylaxis from mast cells, basophils or K-type (Kultschitzky) cells in the pulmonary parenchyma, airways and vasculature. However, it would seem unlikely that 3-MI acts as a hapten, since small amounts of ruminal 3-MI can be demonstrated in healthy cattle, although it is possible that 3-MI is not present in blood in such cases.

From our results, 3-MI given rapidly at doses higher than 1 mg/kg has a more immediate, direct action on bovine lung: the mechanism of this is unknown. Bray, Magnuson & Carlson (1974) have shown that 3-MI intercalates with phospholipid molecules, such as those present in cell membranes and alveolar surfactant. The resultant alterations in cell membrane integrity and alveolar surface tension could result in pulmonary oedema and cell death.

It is interesting that 3-MI has a short plasma half life of about 15 minutes. Carlson *et al.* (1975) have shown that the absorption of oral doses of 3-MI is slow (180 min before peak plasma levels are achieved), so ruminal production of 3-MI from tryptophan would have to be considerable to produce measurable plasma levels of 3-MI. A concentration of approximately 200–300 μg/ml in ruminal fluid would be necessary to maintain a plasma level of 10 μg/ml assuming first-order kinetics for absorption and elimination. It is surprising then, that the levels of 3-MI found in ruminal fluids of cattle dying after being fed L-tryptophan were always low, never rising above 1–10 μg/ml (Yokoyama, Carlson & Dickinson, 1975; Selman *et al.*, 1976). It may be that much higher concentrations are attained in a part of the alimentary tract not sampled. This aspect requires further investigation.

In conclusion, we have shown that 3-MI probably affects the bovine lung directly and not via a metabolite. The immediate clinical signs after rapid 3-MI administration are similar to those seen in experimental anaphylaxis in cattle and this action of 3-MI could well be the result of the release of similar mediators. The half-life of 3-MI in cattle is short (about 15 min) such that marked production of 3-MI from L-tryptophan in the rumen of cattle would be necessary to produce plasma concentrations of the order found after intravenous infusion in these experiments and thus to be considered the causative agent of fog fever.

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