ACUTE RESPIRATORY, CIRCULATORY AND PATHOLOGICAL CHANGES IN THE CALF AFTER INTRAVENOUS INJECTIONS OF THE GALACTAN FROM MYCOPLASMA MYCOIDES SUBSP. MYCOIDES

S. H. BUTTERY, L. C. LLOYD AND D. A. TITCHEN*

CSIRO Division of Animal Health, Animal Health Research Laboratory, Private Bag no. 1, P.O., Parkville, Melbourne, Victoria 3052, Australia, and *Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, Melbourne, Victoria 3052, Australia

IN the course of studies of the development of lesions produced in experimental infection with Mycoplasma mycoides subsp. mycoides (M. mycoides) observations on the influence of polysaccharide substances released by the organism into culture media were made (Hudson, Buttery and Cottew, 1967; Lloyd, Buttery and Hudson, 1971). These substances had systemic effects leading in about two-thirds of cattle to reactions of varying severity in which respiration was increased and coughing occurred, and there was sometimes salivation, incoordination and collapse. In the animals that died there was congestion of the lungs, trachea, bronchi and parts of the alimentary tract; haemorrhage and oedema were seen in lung alveoli (Lloyd et al., 1971).

An extracellular glucan obtained from a mycoplasma strain (L2917) associated with arthritis in cattle (Simmons and Johnson, 1963) caused collapse in cattle (Hudson et al., 1967); an animal that died showed intense congestion of the mucosa of the trachea and bronchi at necropsy. Other reports of acute effects include one by Longley (1951) of collapse from shock in goats and sheep after intravenous injection of effusion from a local mycoplasmal lesion of a goat.

The intoxicant effects of mycoplasma, mycoplasma cellular extracts and whole cultures have been reviewed by Kaklamanis and Thomas (1970).

The present report relates specifically to the effects of the extracellular polysaccharide of M. mycoides. It is obtained as a supernate from the culture medium after removal of the cells by centrifugation. Its pyrogenic activity has been uniformly low or absent. The aim of these investigations in the intact animal was to see if any indication could be obtained of how the material might influence the development of the natural disease produced by M. mycoides, namely contagious bovine pleuropneumonia (CBPP).

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Materials and Methods

The galactan preparations were made as described in detail for galactan F by Hudson et al. (1967) except that the Sephadex G200 column formerly used (80 × 270 mm) was replaced with one 50 × 1000 mm, and that a pneumatically-driven Padberg supercentrifuge was used instead of a steam-driven Sharples supercentrifuge. Three batches of galactan F were made; these were identified as LS30, LS31, LS32. In the case of LS31 the supernate of the culture was heated before and not, as with LS30 and LS32, after acidification with glacial acetic acid; in other respects the procedures for preparation of the three batches were identical.

Control solutions. Uninoculated culture medium (BVFOS) of the same type as that used in the preparation of galactan F was subjected to the same extraction procedures as those used for preparation of the galactan. This provided one of the control solutions injected intravenously into calves. The other control solution used was the 0.85% (w/v) NaCl in which freeze-dried galactan preparations were dissolved for intravenous injection.

Testing for pyrogenic activity. This was done in conscious rabbits (2-6-3-3 kg) by the method described in the British Pharmacopoeia (1968) except that two rabbits were used for each preparation and rectal temperatures were monitored continuously with a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Chemical analyses. Quantitative amino-acid analyses of the galactan preparations were made after hydroiodic acid hydrolysis (Inglis, Nicholls and Roxburgh, 1971). The method has been found suitable for preparations of high carbohydrate content (Nicholls and Inglis, personal communication). Total percentage of protein was calculated as the sum of the individual amino-acid contents of the preparation.

Animal experiments. Most of the experiments were done on 41 calves that were 10-12 weeks old, and weighed 30-90 kg. They were of the Jersey, Hereford and Friesian breeds and crosses of the Jersey and Friesian breeds; the majority were females. All had been taken from their mothers 4 weeks before the experiments and were being maintained on reconstituted skim-milk powder and calf-concentrate pellets. All had begun ruminating. Experiments were undertaken both in conscious and anaesthetised calves.

The experiments on conscious calves were done with the animals standing in a stall, or restrained in lateral recumbency on a table to minimise movement artefacts in electrocardiograms. A venous cannula (Bard, International Ltd, Sunderland) was introduced into one or both jugular veins, and in some experiments a polythene tube was introduced into the pulmonary artery, being passed from the right jugular vein via the right atrium and ventricle. The pressure profiles recorded from these saline-filled tubes on pressure transducers were used to identify the final sites of the tips of the cannulae and verification was obtained at necropsy.

Standard limb leads were used, and recordings made of either lead I, II or III of the electrocardiogram (ECG). Respiratory movements were recorded in conscious calves with the aid of a saline-filled tube introduced via the nasal cavity into the thoracic oesophagus. When chart recordings were discontinued (after periods of up to 30 min.) conscious animals were observed for the possible development of lachrymation and excessive salivation, and for changes in the rate, depth or form of respiration. Sometimes repeated counts of heart rate were made.

Acute experiments were undertaken under anaesthesia induced with halothane and maintained with chloralose (70 mg per kg\textsuperscript{-1}) intravenously or with an aqueous solution of pentobarbitone sodium (60 or 120 mg per ml\textsuperscript{-1}) administered intravenously to effect. A series of observations was made of systemic arterial blood pressure (from a carotid or a tarsal artery), pulmonary arterial pressure and intra-oesophageal pressure (as a respiratory recording), and in some experiments of parotid salivary flow and duodenal or jejunal motility. Conventional recording equipment made by Devices (Welwyn Garden City) was used in conjunction with CEC (Bell and Howell, Basingstoke) pressure transducers.

Observations were also made on one goat anaesthetised with chloralose, a cat decerebrated under halothane anaesthesia, four lambs 1-2 months old (two conscious, one after decerebration and one under pentobarbitone sodium anaesthesia), 16 conscious rodents (five guinea-pigs, six mice, three rabbits and two rats) and 12 rats maintained under urethane anaesthesia.

All preparations tested for systemic effects were injected intravenously.
5-Hydroxytryptamine injections. As the effect of galactan on calves in some respects resembled that of 5-hydroxytryptamine (5-HT) on calves (Aitken and Sandford, 1972) it appeared desirable to make a direct comparison of the two phenomena under the existing experimental conditions. Injections were made intravenously while the calves were under anaesthesia, and recordings of blood pressures and respiration were made as described above for the galactan experiments.

Haematology. Blood samples were withdrawn from the pulmonary or carotid artery or the jugular vein by means of polythene or polyvinyl tubes, with or without inner silicone rubber linings. The blood was taken into tubes with dried ethylene diamine tetracetic acid (EDTA) for total and differential WBC and platelet counts and into Hellar and Paul anticoagulant mixture for determination of the packed cell volume (PCV). Platelet counts were carried out according to the method of Dacie and Lewis (1968) in which samples were diluted in ammonium oxalate solution, and the PCV was determined as described by Schalm (1965). An analysis of variance was calculated as described by Bliss (1967).

Skin test. The aim was to seek evidence of immunological sensitisation to attempt to explain the effect of galactan. Approximately 3 min. before and 3 min. after the intravenous injection of 10 ml of 1 % (w/v) Evans blue, calves received intradermal injections of 0-1-ml volumes of three solutions containing galactan in concentrations of 10, 100 and 1000 pg per ml and of a fourth solution containing NaCl 0.85 % (w/v). The injection sites were in the unpigmented skin in the caudal ventral region of the lateral abdominal wall. Blue areas that developed within 30 min. were measured and any area that exceeded by 2 mm or more in diameter the area associated with the saline control inoculum was regarded as positive.

Serology. Samples of sera from eight calves were tested for complement-fixing (CF) antibody by the method of Campbell and Turner (1953) as modified by Etheridge and Lloyd (1968). The eight sera were also tested for their ability to potentiate complement fixation (Cottew, 1962). The amount of complement fixed in the reaction between a standard concentration of galactan and the heated serum under test was determined in the presence of unheated potentiating serum. The result was expressed as the number of 50% complement units fixed. The indirect haemagglutination test was carried out by the method of Cottew (1960).

Pathological procedures. Necropsy was carried out immediately after the animals were killed, special but not exclusive attention being given to the respiratory and alimentary tracts. Specimens from these were taken, as appropriate, into 10 % buffered formalin and Zenker acetic-acid fixatives and stained with haematoxylin and eosin (H and E), azure eosin (AE), phosphotungstic acid haematoxylin (PTAH), Verhoeff’s and Van Gieson’s stains. In two animals the lungs were perfused, via the pulmonary artery, first with physiological saline and then with Kaiserling’s fixative solution; the perfusion pressure was limited to a maximum of 90 cm of water.

Animals were killed at the end of acute experiments either by excess pentobarbitone sodium, or by exsanguination or by a captive-bolt pistol. Conscious animals were all killed with the pistol. Precautions taken to ensure that blood, saliva and ingesta did not enter the lungs at slaughter included leaving the endotracheal tube in position in animals killed with pentobarbitone sodium and immediately isolating and tying the oesophagus and trachea in those that had been shot.

RESULTS

Reactions to galactan injection

Respiratory and cardiovascular changes. In 20 of 28 calves tested, a single dose of 100 μg per kg⁻¹ of the galactan preparations LS30 or LS32 had severe effects on respiration and blood pressure within 25–30 s of its injection into either a recurrent tarsal or a jugular vein. Eight of nine conscious calves and 12 of 19 anaesthetised calves showed these effects (table 1). The initial reaction consisted of a deep inspiratory movement followed by cessation of
respiration for 30–60 s. Respiration recommenced at a much greater depth than before injection and, within 120 s of the initial reaction, was regular but at about twice the resting rate. This continued for 30 min. or more after the galactan injection. Other animal species were tested at the same dose rate. Of these, the goat showed a profound and immediate reaction and died 9 min. after the injection, two conscious lambs coughed severely from about 2 min. afterwards, and the mice, guinea-pigs, rabbits, rats, anaesthetised lamb and decerebrated cat and lamb did not react.

Blood-pressure recordings made in the 12 anaesthetised calves that reacted to galactan (table I) showed that the first effect on systemic arterial blood pressure coincided with the initial deep inspiration, and there was a slowing of the heart for a few seconds. Over the next 30 s a marked vasodepression developed, continued for 120 s and was succeeded by a return first towards, and then above, the resting blood pressure level (fig. 1). At the time of elevated blood pressure the heart rate was increased.

**Table I**

*Description, state of consciousness and response of calves inoculated intravenously with galactan*

<table>
<thead>
<tr>
<th>Calf number</th>
<th>Breed</th>
<th>Sex</th>
<th>Conscious (C) or anaesthetised (A)</th>
<th>Response to galactan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fr</td>
<td>F</td>
<td>A</td>
<td>+</td>
</tr>
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<td>2</td>
<td>X</td>
<td>F</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>Fr</td>
<td>F</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Fr</td>
<td>F</td>
<td>A</td>
<td>+</td>
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<tr>
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<tr>
<td>7</td>
<td>J</td>
<td>F</td>
<td>A</td>
<td>+</td>
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<tr>
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<td>Fr</td>
<td>F</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
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</tr>
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<td>F</td>
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<td>+</td>
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<td>F</td>
<td>C</td>
<td>+</td>
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<td>F</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
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<td>F</td>
<td>C</td>
<td>+</td>
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<td>M</td>
<td>A</td>
<td>+</td>
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<td>H</td>
<td>M</td>
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<td>—</td>
</tr>
<tr>
<td>20</td>
<td>H</td>
<td>M</td>
<td>A</td>
<td>+</td>
</tr>
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<td>—</td>
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<td>24</td>
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<td>X</td>
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<td>C</td>
<td>—</td>
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<td>H</td>
<td>F</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>H</td>
<td>F</td>
<td>C</td>
<td>+</td>
</tr>
</tbody>
</table>

Fr = Friesian, X = Friesian cross breed, J = Jersey, H = Hereford.
F = Female, M = male.
+ = Respiration ceased, pulmonary arterial blood pressure rose and, where measured, carotid arterial blood pressure fell.
* This table does not refer to inactive batch LS31.
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Pulmonary arterial blood pressure started to rise at the time of the single deep inspiratory effort and when the slowing of the heart was at its most striking. After an initial rise in both systolic and diastolic pulmonary arterial blood pressures, the systolic pulmonary arterial pressure fell and for 120 s there was virtually no pulse pressure. Such a response in one experiment together with a test confirming the patency of the recording cannula is shown in fig. 1.

In the remainder of the paper a reacting calf is taken to be one in which respiration ceased, pulmonary arterial pressure rose and, where measured, the carotid arterial pressure fell after galactan was injected.

Pathological changes. The lungs of reacting animals, as defined above, showed four changes. The first of these was haemorrhage. This was seen consistently, even when the other changes—oedema, capillary involvement and distension of distal airways—were not present. It occurred in discrete foci in lung parenchymatous tissue (fig. 2) and in the adventitia of medium-sized vessels (fig. 3); red cells were present in the distended lymphatics in the interlobular septa. Where a point of origin could be determined, the haemorrhages in the parenchyma were associated with alveolar ducts but not with arteries or veins. In those lungs in which oedema occurred, haemorrhages were usually in the oedematous area.

Oedema, the second change, was apparent as a doughy consistency of the pulmonary tissue, distended interlobular septa and rounded borders of lobes. Histologically there were foci, varying in size, of fluid-filled alveoli (fig. 4), distension of perivascular and peribronchial lymphatics and connective tissue and distended lymphatics in the interlobular septa. In mildly affected calves,
signs of oedema were seen only in interlobular, perivascular and peribronchial lymphatics.

The third change was seen as material strained with PTAH in the capillaries in areas of oedema and haemorrhage (fig. 5). It was still present after perfusion, suggesting that it was firmly attached to vessel walls and was probably formed before the animal was killed. The lesion was identified as capillary thrombosis.

A large proportion of the lungs from affected animals showed the fourth change—dilation of the distal parts of the airways (fig. 2). In some lungs, the alveolar ducts in particular were widely distended, but adjacent were areas where the airways were contracted or no longer visible and the respiratory tissue was partially or completely collapsed.

The changes in the lungs of the goat were similar to, but more striking than, those in the calves.

*Packed cell volume.* In seven of a group of eight anaesthetised calves in which particular care was taken to collect a series of blood samples, significant differences were noted between the means of the PCV of samples collected before and after injection of galactan (table II). The mean of the samples collected 5 min. after injection was significantly different ($P<0.05$) from that of the pre-administration samples; those at 15 and 30 min. were highly significantly different ($P<0.01$). For calf no. 7 the PCV after galactan was injected was not significantly different from its initial value; the pre-injection PCV was lower than that of the other animals and this may have indicated some degree of abnormality.

*WBC count.* The total WBC count did not follow the pattern of the PCV in the 10 anaesthetised calves from which counts were made; it was either maintained at its original level or it fell after the galactan was injected. In subsequent experiments we found that this was caused by the chloralose

**Table II**

*The effect of intravenous injection of galactan on the packed cell volume in calves*

<table>
<thead>
<tr>
<th>Calf number</th>
<th>Packed cell volume (%)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before injection</td>
<td>5 min. after injection</td>
<td>15 min. after injection</td>
<td>30 min. after injection</td>
</tr>
<tr>
<td>1</td>
<td>41.7</td>
<td>42.6</td>
<td>43.5</td>
<td>42.7</td>
</tr>
<tr>
<td>2</td>
<td>41.2</td>
<td>43.1</td>
<td>43.8</td>
<td>47.7</td>
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<tr>
<td>3</td>
<td>42.2</td>
<td>47.5</td>
<td>46.9</td>
<td>44.2</td>
</tr>
<tr>
<td>4</td>
<td>35.6</td>
<td>37.6</td>
<td>37.4</td>
<td>40.1</td>
</tr>
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<td>5</td>
<td>36.4</td>
<td>37.8</td>
<td>38.7</td>
<td>37.0</td>
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<td>44.5</td>
<td>43.8</td>
<td>43.0</td>
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<td>33.2</td>
<td>33.2</td>
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<td>41.0</td>
<td>41.7</td>
<td>41.9</td>
<td>41.8</td>
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<td>(39.1)</td>
<td>(40.9)*</td>
<td>(41.2)†</td>
<td>(41.2)†</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from the pre-injection mean ($P<0.05$).
† Highly significantly different from the pre-injection mean ($P<0.01$).
Mean values are given in parenthesis.
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anaesthesia. This, together with inconsistent results in differential counts in four animals, led us to discount the effect of galactan on WBC counts.

Platelet count. There was little change in platelet counts in two conscious and one anaesthetised calf each treated with galactan.

Variability of galactan preparations. Preparation LS31 did not produce reactions in any of two anaesthetised and four conscious calves tested.

Variability of experimental calves. Friesian crossbred animals had the highest proportion of non-reactors (P<0.05). There was no significant difference in the ratio of non-reactors to total number in anaesthetised and conscious groups. The effect of sex could not be tested as it was confounded with breed (see table I).

Reactions to control solutions

Neither the saline used to dilute the galactan, nor the product extracted from the BVFOS medium by the same method as that used to extract the galactan from cultures of M. mycoides grown in BVFOS, produced responses. The volume of each control solution was the same as the volume of galactan preparation injected into that animal.

Reactions to second doses of galactan

Second doses of galactan of 100–500 µg per kg⁻¹, i.e., the same dose or up to five times the first dose, were given to nine anaesthetised calves, seven of which had previously reacted. The doses were repeated in one calf 135 min. and in the other eight 60 min. after the first. None showed any response to the second dose.

5-Hydroxytryptamine

Each of seven anaesthetised calves given 5-HT, 100 µg per kg⁻¹ intravenously, showed falls in systemic arterial blood pressure and rises in pulmonary arterial pressure. Respiration was slowed or stopped. The effects lasted for 20–100 s and could be produced a second time in the same animal. The effects were reduced in two calves treated with methysergide, 40 and 100 µg per kg⁻¹. Aitken and Sanford (1972) made similar observations. In four of five animals in which platelet counts were carried out a striking fall was detected 30–120 s after injection. This reaction was different from that produced by galactan. Histopathological examination revealed haemorrhages at the alveolar duct level but no oedema in the lungs of animals that received 5-HT.

Skin test for immediate hypersensitivity to galactan

There was no relation between the skin-test result and physiological reaction to intravenous injection of galactan in a total of 14 calves tested for their response to galactan under anaesthesia. Three animals with negative skin tests and one with a positive skin test did not react to intravenous galactan, three animals with negative skin tests and one with a positive skin test reacted
mildly and four animals with negative skin tests and two with positive skin tests reacted strongly.

The diameter of the blued area at the control saline-injection sites ranged between 0 and 7 mm. Positive reactions occurred only at the sites of injection of 10 μg and 100 μg galactan.

**Complement-fixation and indirect-haemagglutination tests**

Eight calf sera were tested; three were from calves that did not react to galactan and five were from animals that reacted. All sera enhanced complement fixation by a known positive serum in the presence of complement and galactan. There was no significant difference between the degree of enhancement produced by sera from reacting and non-reacting calves. None of the eight sera fixed complement in the presence of galactan and an enhancing calf serum.

The three sera from non-reactors and two from reactors did not agglutinate galactan-coated red cells. One of the other sera caused agglutination at a dilution of less than 1 in 80, while the other two caused agglutination at dilutions greater than 1 in 80.

**Analysis of galactan preparations**

The protein contents of preparations LS30, LS31 and LS32 were 6·2, 4·6 and 6·6 % (w/v) respectively. No significant differences were detected between the content of individual amino acids in the preparations.

**Pyrogenicity tests on galactan**

Two galactan preparations that elicited systemic reactions (LS30 and LS32) gave immediate temperature increases in pairs of rabbits ranging from 0·4°C–0·7°C. The preparation that produced no systemic reactions (LS31) caused temperature increases of 0·2°C and 0·6°C. All animals remained alive and healthy for the next 7 days.

**DISCUSSION**

The most plausible explanation for the changes described is that the galactan exerted specific effects on the vascular system of the lung and on respiration. There was evidence that vaso-active agents such as 5-HT were implicated but there was no support for the hypothesis that it was an immunological phenomenon. Some of the pathological changes that appeared after the injection of galactan resembled changes seen in natural cases of CBPP.

The rise in the pulmonary arterial blood pressure in the calves that reacted indicated that the injection of galactan caused an increase in the resistance to blood flow through the pulmonary vascular bed. This probably arose from pulmonary venous constriction, perhaps resulting from contraction of the fibromuscular tissue present in the walls of pulmonary veins in cattle (Best
FIG. 2.—Lung of galactan-treated calf, photographed with H (now 45) Wratten filter, showing focal haemorrhages (arrowed) and distension of distal parts of airways. Azure eosin (AE). ×110.

FIG. 3.—Haemorrhages (arrowed) in the adventitia of an artery in the same lung as that in fig. 2, also photographed with H (now 45) Wratten filter. AE. ×320
FIG. 4.—Oedema in the lung of a calf treated with galactan. To the right of the vertical interlobular septum the alveoli are distended and fluid-filled; the interlobular septa are moderately distended. Small deposits of fibrin are randomly distributed in both oedematous and non-oedematous areas. Phosphotungstic acid haematoxylin (PTAH). ×110.

FIG. 5.—Capillary thrombosis and haemorrhage in the lung of a calf treated with galactan. The fibrin deposits (arrowed) are present in capillaries; masses of red blood cells are apparent in alveoli and alveolar walls. PTAH. ×560.
and Heath, 1961). The fall in systemic arterial blood pressure in our experiments confirmed that there was decreased venous return to the left atrium.

An initial effect of this kind might explain some of the pathological changes. The haemorrhages at the alveolar duct level, which were also present in calves given injections of 5-HT, probably arose from hypertension in the pulmonary arteries. It is these arteries, not the bronchial artery, that supply blood to the region in question (Miller, 1947). Capillary hypertension, the possible release of biogenic amines (see below) and the continued laboured respiration could all have contributed to the production of haemorrhages by galactan.

The pulmonary oedema was due, at least in part, to the increased resistance to pulmonary blood flow. The increase in intrapulmonary pressure would, as shown by Guyton and Lindsey (1959), cause fluid to accumulate. Visscher, Haddy and Stephens (1956) listed indirect determinants of pulmonary oedema such as hypoxia with heart overload or failure, compression of pulmonary veins, arterial hypotension, vasoactive agents such as histamine and both sympathomimetic and parasympathomimetic agents; direct determinants included elevated capillary blood pressure and increased capillary permeability. Any of these may have played a role in promoting pulmonary oedema in our experiments.

The capillary thrombosis was probably due to the same vascular phenomenon. As a result of the increased resistance to flow of blood through the veins, the flow through the capillaries would be slowed; sludging and then clotting would occur, and thrombi would develop.

The reactions of calves treated with galactan were similar in some respects to those of calves treated with biogenic amines. In our experiments 5-HT and galactan caused similar haemorrhages and changes in blood pressure, but the two substances differed in respect of the production of oedema and of change in platelet count. Aitken and Sanford (1972) showed that 5-HT caused alteration in pulmonary arterial blood pressure and systemic vasodepression, which they associated with stimulation of the contractile elements of the pulmonary vascular bed. Wray and Thomlinson (1969) implicated 5-HT in the development of pulmonary vascular changes in anaphylactic reactions in cattle in which there was oedema of the gut and severe gastrointestinal signs but no pulmonary oedema. They used doses of protein 5000 times greater than those injected in the present experiments.

The galactan also had both rapid and enduring effects on respiratory movements. The initial deep inspiratory effort (followed by apnoea) accompanied by a brief bradycardia has some features in common with reactions encountered on intravenous or intra-atrial injection of veratridine or phenyl diguanide. The importance of pulmonary and cardiac receptors in such reactions has been recognised and discussed by Dawes and Comroe (1954) and Paintal (1973). The later and continuing increased rate and depth of respiration was probably a reflexion of the development of pulmonary oedema and the sensitisation of pulmonary receptors. This has been postulated as occurring in reactions to high altitude in man and in the experimental pulmonary oedema of animals produced by alloxan.
The failure of preparation LS31 to cause reactions in any of the calves is attributed to differences in preparation. The partially purified extracts of LS31 were heated at 100°C at pH 7.0 instead of pH 5.0 as for LS30, LS32 and the other preparations made as described by Lloyd et al. (1971). It would seem that an exact form of extraction is required if an active preparation is to be obtained and this contrasts with the requirements for the preparation of pyrogens.

It seems unlikely that the effects of the preparations were due to pyrogens as their stability would not have been affected by the conditions that led to the destruction of the active principle in the galactan preparation LS31. The criteria applied by the *British Pharmacopoeia* indicate that the test preparations used were weakly if at all pyrogenic, even though the rabbit tests were conducted with 10 times the dose used to produce circulatory and respiratory reactions in calves.

Similarly, there was no evidence to suggest that the reactions described had an immunological basis. The indirect haemagglutination test, the serum-enhanced complement consumption test and a cutaneous test for immediate hypersensitivity did not demonstrate any significant differences between animals that showed changes in blood pressures and respiration and those that did not. These observations lead us to postulate that the galactan occupied receptor sites on either blood elements or blood-vessel components and caused the release of biogenic amines locally in the lung. The sensitivity of the calves would then depend on the availability of receptor sites. The well established release of 5-HT from platelets (Zucker and Borelli, 1955) and the implication of platelets early in the pathogenesis of CBPP (Lloyd, Piercy and Bingley, 1975) suggest the receptor sites are on platelets.

The failure of animals to react to a second dose of galactan can be explained in one of three ways. It is possible that the galactan caused a release, to the extent of depletion, of a cell-bound mediator on, say, blood platelets. A second possibility assumes a long-term binding between a releasing factor (galactan) and a cell receptor so that the receptor was not available for a second dose of galactan. Galactan has been shown to remain bound to erythrocytes for the duration of their survival in in-vitro experiments (Buttery, unpublished observation). The third possibility is that the response was an immediate-type hypersensitivity reaction, such as anaphylaxis, in which after the initial reaction the animal was desensitised and no longer reactive to a second dose. This possibility is discounted because, as already stated, there was no positive evidence of immunological involvement.

No explanation can be given for the different reactivity of the Friesian cross-bred calves. Possibly non-reactors, which made up 29% of those tested, were animals that were resistant to *M. mycoides*, but it is generally considered that European breeds are uniformly susceptible to CBPP. Turner (1954) found that 25% of cattle exposed to infective animals did not develop the disease. He did not report different breed susceptibilities.

What is the role of the galactan in the pathogenesis of CBPP? It produces oedema characteristic of the natural disease (Lloyd and Trethewie, 1970). It
may also play a part in the formation of thrombi. Lloyd et al. (1975) have shown that, in cattle with developing CBPP, changes occur in platelet numbers and blood-clotting factors, indicating that thrombosis is taking place before exudation, pneumonia and lymphatic involvement. They postulated that there must be a local effect in the lung to account for localisation of the thrombi in that site. Contraction of fibromuscular tissue associated with smaller pulmonary vessels may lead to this local effect.

This paper provides evidence that the galactan has an effect on pulmonary blood vessels. In the case of an infection with CBPP this might be manifested either as a general effect where galactan is conveyed to the whole lung by the circulation, or as a local effect where it only operates on vessels in a circumscribed area. If the galactan is distributed by the circulation, the amount required could only be produced by a substantial lesion. This is not consistent with evidence that thrombosis occurs early in the disease process, perhaps before inflammatory lesions are detected (loc. cit.). An alternative hypothesis, that the effect is exerted locally due to diffusion of galactan from a focus of infection, is more attractive. Under these conditions a local concentration sufficient to constrict vessels could be achieved in a small section of the lung. These experiments do not provide evidence as to whether galactan in an extravascular location will have the same effect as when it is injected intravascularly.

**SUMMARY**

Twenty of 28 calves, 10–12 weeks of age when given intravenous injections of the galactan from *Mycoplasma mycoides* subsp. *mycoides*, showed transient apnoea, increased pulmonary arterial and decreased systemic arterial blood pressures, and increased packed-cell volume. Necropsy revealed haemorrhages associated with alveolar ducts and vessel walls, areas of pulmonary oedema, usually associated with the haemorrhages, dilated airways and, in some, capillary thrombosis. Animals that had shown changes in blood pressure and respiration in response to a dose of galactan did not react to a second dose an hour later. One goat tested died, four lambs were mildly affected and a cat and several rats and guinea-pigs did not respond.

It is suggested that the galactan released biogenic amines that produced the effects listed. Immunological mechanisms were discounted on the grounds that only a small amount of antigenic material was injected at the time the reaction occurred, and neither serological nor skin tests produced any evidence of prior sensitisation to the galactan or a similar substance.

A relationship between reactivity to the galactan and susceptibility to the natural disease has been suggested. This, together with the pulmonary oedema found in galactan-treated calves and in natural lesions of contagious bovine pleuropneumonia (CBPP), and the possibility that contraction of blood vessels could be an initiating cause of thrombosis indicates the role that galactan may play in the pathogenesis of CBPP.

Mr A. S. Inglis from the CSIRO Division of Protein Chemistry made the amino-acid analyses and Mr J. Patterson of the Department of Veterinary Preclinical Sciences, University
of Melbourne, assisted with the instrumentation and recording. At the CSIRO Animal Health Research Laboratory, Mr G. S. Cottew made the indirect-haemagglutination tests and Mr J. R. Etheridge the complement-fixation tests. Mr A. Rowlett was responsible for the histological preparations, Mr M. Kalade assisted with the haematology, Mr L. Harrop with the chemical preparations and Mr B. Heggie with the serology and microbiology. We thank all these people for their generous contributions to the project.

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