THE EFFECT OF THE GALACTAN AND OTHER ANTIGENS OF *MYCOPLASMA MYCOIDES* VAR. *MYCOIDES* ON EXPERIMENTAL INFECTION WITH THAT ORGANISM IN CATTLE

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MYCOPLASMA MYCOIDES VAR. *MYCOIDES* (hereafter referred to as *M. mycoides*), the causal organism of contagious bovine pleuropneumonia (CBPP), synthesises a galactan of unusual structure consisting mainly of galacto-furanosyl units in 1-6 β linkage (Plackett and Buttery, 1964). The galactan forms a slime layer (Gourlay and Thrower, 1968) and comprises about 10 per cent. of the dry weight of cells in young cultures. During growth it is released into the culture fluid, where it accumulates in large amounts, and it is also present in the blood-stream of infected cattle at certain stages of the disease (Turner, 1962).

Galactan has been isolated from the cells of *M. mycoides* (Buttery and Plackett, 1960) and also from the culture medium supernatant after growth of the organism (Hudson, Buttery and Cottew, 1967).

Polysaccharide prepared from the washed cells of *M. mycoides* by phenol extraction contains a major component P and a number of minor components detectable by immunodiffusion (Plackett, Buttery and Cottew, 1963). It has been termed galactan C (Hudson *et al.*). If the cells are extracted with chloroform-methanol before being extracted with phenol, the resulting polysaccharide, which we call L, contains the major component P and one minor component detectable by immunodiffusion. Several antigens can be recovered from the chloroform-methanol extract by washing it with potassium chloride solution. This material is called W in the present paper.

Polysaccharide isolated from the culture medium supernatant contains a major component behaving like component P in serological tests, one minor component detectable by immunodiffusion and some non-specific material derived from medium constituents. This polysaccharide has been termed galactan F (Hudson *et al.*).

The infection that develops after a subcutaneous injection of a moderately virulent strain of *M. mycoides* into normal susceptible cattle causes a large swelling at the site of injection, and a mycoplasmaemia of moderate duration, but no joint lesions. When cattle were given parenteral injections of galactan F from the V5 strain and infected with a strain of *M. mycoides* of moderate virulence, prolonged mycoplasmaemia and joint lesions resulted (Hudson *et al.*). This occurred when a total dose of 2 mg per kg of galactan was given over 5 days. The effect of doses lower than this, of galactan from other strains both virulent and avirulent, of the fractions of galactan C on infected cattle and the effect of galactan F on immune cattle are reported here. The effect of

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the galactan on the localisation of *M. mycoides* infection in the pleural cavity and lungs of cattle with subcutaneous infection was also studied.

Hudson *et al.* found that inoculation of galactan F and Freund's adjuvant did not protect cattle against infection with *M. mycoides*. As galactan F and W between them contain all the antigens that have been extracted from *M. mycoides* in this laboratory, the production of antibodies to the W fraction and their influence on the susceptibility of cattle to CBPP were also investigated.

The mycoplasmaemia apparent after treatment of animals with galactan and infection with *M. mycoides* suggested that blood clearance mechanisms were affected by the galactan. Organisms were injected intravenously into cattle after galactan treatment to determine whether the galactan inhibited the clearance mechanisms.

Finally, further observations were carried out on the sudden collapse observed in some cattle when galactan was injected intravenously (Hudson *et al.*).

MATERIALS AND METHODS

Preparation of galactan F. Galactan F was prepared from the supernatant of cultures of *M. mycoides* after removal of the cells (Hudson *et al.*). The strains of *M. mycoides* from which galactan F was isolated were V5 (Turner, 1959), KH3J (Report, 1960) and Gladysdale (Turner, 1961).

Preparation of fraction W. Cultures of *M. mycoides*, strain V5, grown in 15-litre batches of supplemented BVF-OS medium (Plackett *et al.*) were centrifuged in a steam-driven Sharples supercentrifuge. The cells from each batch were resuspended in 80 ml of 0.2M-NaCl and resedimented at 40,000*g* for 15 min. They were washed once in cold distilled water and freeze-dried.

The combined yields from four batches were resuspended in 100 ml 0.1M-KCl; 600 ml of chloroform-methanol (1:1 v/v) was added and the mixture was agitated intermittently in a Waring Blender for 20 min. The solid residue was then filtered off and extracted twice with 100-ml quantities of chloroform-methanol (2:1 v/v). The extracts were combined, 150 ml of 0.1M-KCl was added to them and they were mixed thoroughly and left overnight.

The material separated into two clean phases plus an interfacial gel. The clean upper phase was combined with the upper phase obtained by centrifuging the interfacial gel, pervaporated to 40 ml and dialysed against saline. This material was fraction W. Such preparations have been found to contain lipo-oligosaccharides. Up to three lines appeared in immunodiffusion tests with this fraction and selected antisera from cattle that had been experimentally infected with *M. mycoides*.

Preparation of fraction L. The solid residue from the chloroform-methanol extraction was extracted for 5 hr with boiling methanol and then ground to a fine powder, which was suspended in 300 ml of distilled water and extracted with an equal volume of aqueous phenol (phenol-water 6:1 w/w) for 45 min. at 65°C. The mixture was placed in the cold room overnight and then centrifuged at 4°C. The upper aqueous phase was removed and dialysed against changes of 0.15M-NaCl to remove phenol. This material was fraction L. Immunodiffusion of fraction L and galactan F against bovine antiserum gave one strong line with the fraction L and this line fused completely with the line given by galactan F. A second, minor line sometimes appeared.

Dry weights of the above preparations were determined on material dialysed against distilled water. Samples of known volume were dried to constant weight at 100°C in small vials.

Experimental cattle. These comprised: young dairy cattle aged between 6 and 19 mth reared on the laboratory's field stations; culled dairy cattle most of which were at least 42 mth old when purchased at markets; and a few beef cattle 34 mth old. They were weighed,

bled for serology and examined for lameness before use. Where relevant, the ages of cattle used in each experiment are given in the preamble to the results. Unless otherwise stated, they were all susceptible to *M. mycoides* infection; they had not been exposed to the disease nor had they been vaccinated.

Experimental infections. Subcutaneous infections were produced by injecting 1 ml of a 48-hr filamentous culture of the V5 strain of *M. mycoides*. The number of organisms, of the order of 10^9 viable units per ml, was determined by the method of Miles and Misra (Miles, Misra and Irwin, 1938). The same strain was used in the experiment on the clearing of organisms from the bloodstream.

To study the effect of the galactan on subcutaneous *M. mycoides* infection the total dose was divided on every occasion into three parts; half was given intravenously 6 hr before the subcutaneous injection of *M. mycoides*, one-quarter was given 48 hr after the first inoculation and the final quarter 120 hr after the first inoculation.

The resistance to infection of the cattle vaccinated with the W fraction of the galactan was tested in a "natural exposure" experiment (Hudson and Turner, 1963) in which the Gladysdale strain of *M. mycoides* (Turner, 1961) was used to infect the donor animals by endobronchial intubation.

Serology. Complement fixation (CF) tests (Campbell and Turner, 1953) with modifications described by Etheridge and Lloyd (1968) and serum agglutination (SAB) tests (Turner and Etheridge, 1963) were carried out on blood samples taken from the cattle at regular intervals.

Blood culture. Animals were bled at regular intervals and 5 or 10 ml of blood inoculated into either 45 or 90 ml (10 per cent. blood) of BVF-OS broth containing penicillin and thallium acetate (BVF-OS—PTA) (Turner and Trethewie, 1961). Cultures were incubated at 37°C and were examined by dark-ground illumination each day for 7 days to determine whether organisms were present.

Necropsy procedure. Necropsies were carried out on animals that died or were killed *in extremis*. Those that recovered were slaughtered at abattoirs and only a limited necropsy was possible. Particular attention was paid to the thoracic cavity, lungs and associated lymph-nodes, kidneys and limb joints. Material for culture was taken from the subcutaneous lesion, prescapular and precutaneous lymph-nodes, lungs and associated lymph-nodes and abnormal kidneys and limb joints and sown into BVF-OS—PTA broth. Specimens for section were taken from the subcutaneous lesion and lesions in lungs, lymph-nodes, joints and kidneys, fixed in 4 per cent. buffered formaldehyde or in Zenker-acetic and stained as a routine with haematoxylin and eosin (HE). Occasional sections were stained with Lillie's azure-eosin (Lillie, 1965) and phosphotungstic acid-haematoxylin (PTAH).

Scoring procedure. An arbitrary scoring system has been devised (Hudson and Turner) to integrate measurements of various aspects of pleuropneumonia infections. The serological response and the clinical response are graded from 1 to 3. The necropsy findings take into account the presence of adhesions and lesions and whether *M. mycoides* is isolated; these lesion scores are then multiplied by factors of 2 or 3 depending on the size of the lesion. Thus the maximum score for the serological and clinical response is three in each case, the maximum pathology score is 12, and the maximum over-all score is 18.

RESULTS

Effect of dose rate of galactan F from strain V5 and of galactan F from various other strains of M. mycoides on the course of subcutaneous M. mycoides infection in cattle.

Galactan from the V5 strain was injected into four cattle at a total dose of 0.66 mg per kg (one-third of the total dose used in the previously reported experiments of Hudson *et al.*), and four cattle received 0.22 mg per kg (one-ninth of the previously reported dose). Each animal was also given a

subcutaneous injection of 1 ml of a filamentous culture of strain V5. The animals were from 6 to 18 mth old. The results are given in table I.

TABLE I

Effect of reduced doses of galactan F prepared from strain V5 and full doses of galactan F prepared from the more virulent Gladysdale strain and from the avirulent KH3J strain on the course of infection provoked by the subcutaneous inoculation of the V5 strain of M. mycoides in cattle

Group given	Animal no.	Period to slaughter (days)	Joint lesions	Lung lesions	Blood cultures positive†
V5 galactan F ($\frac{1}{9}$ dose: 0.22 mg per kg)	S19	48	...	...	5/15
	S27	48	...	...	1/13
	S40	48	...	...	1/13
	S48	48	...	...	5/13
V5 galactan F ($\frac{1}{3}$ dose: 0.66 mg per kg)	S17	48	...	...	1/13
	S33	48	...	Pleurisy* +ve	11/14
	S36	48	...	...	4/13
	S96	146	...	...	8/13
Gladysdale galactan	S97	48	...	...	7/13
	S39	146	...	...	6/13
	S28	48	R. humero-scapular* —ve	...	10/14
	S26	48	...	Pleurisy* +ve	13/14
KH3J galactan	S13	48	...	...	7/13
	S30	48	...	...	7/13
	S38	26	L. humero-radial* +ve L. tarsus* +ve	...	6/10
	W1	31	...	...	11/12
No galactan	S85	48	...	...	0/13
	S43	146	...	...	1/13
	S44	182	...	...	2/13
	S23	183	...	...	3/13

* Culture from lesion positive or negative for *M. mycoides*.

† Number of days on which cultures were positive over the number of days on which samples were taken.

... = None found.

Of the four that received one-third of the usual dose, two showed a high proportion of positive blood cultures and two did not. There were no joint lesions, but one of the animals with a high proportion of positive blood cultures showed extensive pleurisy. Those treated with one-ninth of the usual dose were similar to the controls; they had few positive blood cultures and there were no lesions in the joints or thorax.

To test the efficacy of galactan F from other strains of *M. mycoides*, four

cattle were given intravenous injections of galactan from the Gladysdale strain, a strain of high virulence, and four were given galactan from the KH3J strain, an avirulent strain; with both preparations, the dose was 2.0 mg per kg. These eight cattle together with four untreated control cattle were each given subcutaneous injections of 1 ml of a culture of the V5 strain. The animals were from 6 to 19 mth old. The results are included in table I.

The cattle treated with galactan F from the Gladysdale strain had a high proportion of positive blood cultures. One animal showed arthritis and tenosynovitis of the right humero-scapular joint. One other animal had extensive pleurisy. Those treated with the KH3J strain galactan F also had a high proportion of positive blood cultures and one animal developed arthritis and tenosynovitis of the left humero-radial joint and the left tarsus.

Effect of the galactan F on infection of immune cattle with M. mycoides

The effect of the galactan on the persistence of mycoplasmaemia and the development of joint lesions was tested in immune cattle. Four animals, about 20 mth old, were used. These had been given injections of the V5 strain of *M. mycoides*, had recovered and had then proved resistant to challenge with the Gladysdale strain by the subcutaneous route. Two were each given intravenous injections of pooled galactan from the Gladysdale, V5 and KH3J strains at a total dose of 2 mg per kg body weight and a subcutaneous injection of 1 ml of a filamentous culture of the V5 strain.

There were no positive blood cultures and no joint lesions in either the galactan-treated or the untreated immune cattle.

Effect of the fractions of galactan C on the course of subcutaneous M. mycoides infection in cattle

L fraction and galactan F were each injected intravenously into four cattle at a total dose rate of 2.0 mg per kg. W fraction was injected intravenously into four cattle at a dose rate that bore the same relation to the dose of the L fraction as did the amount of W fraction to the amount of L fraction in the whole organism, namely in the ratio of 1:12. All eight cattle and four control animals each received 1 ml of a filamentous culture of the V5 strain of *M. mycoides*. The animals were all 9 to 18 mth old.

The detailed results are given in table II. The effects of treatment with the L fraction were similar to those with the galactan F; some of the cattle had a prolonged mycoplasmaemia and one in each group developed synovitis and arthritis. One animal in the group treated with galactan F developed pleurisy. The subcutaneous lesion behind the shoulder was small and circumscribed and the pleurisy was not due to direct extension into the pleural cavity.

In contrast, all those in the group that received W fraction had a low proportion of positive blood cultures and none developed a joint lesion, the results being similar to those of the control group. It appeared that W fraction, in the dose used, had little or no effect.

*Effect of galactan from M. mycoides on the localisation of
M. mycoides infection in the pleural cavity and lungs of adult cattle*

The three cattle that developed pleurisy in the foregoing experiments had been given an injection of galactan before and after a subcutaneous injection

TABLE II

Comparison of the effects of fractions L and W of cellular galactan and of supernatant galactan F of M. mycoides on the course of infection provoked by the subcutaneous inoculation of the V5 strain

Group given	Animal no.	Period to slaughter (days)	Joint lesions	Lung lesions†	Blood cultures positive§
Cell fraction L	A75	34	Nil	Nil	7/8
	A77	34	Nil	Nil	2/8
	S95	33	R. acetabular* +ve	Nil	4/8
	W32	34	Nil	LDL* -ve	7/8
Cell fraction W	A76	34	Nil	Nil	2/8
	M49	34	Nil	Nil	3/8
	W21	34	Nil	LDL* -ve	2/8
	W46	34	Nil	RDL* -ve Nil	1/8
Supernatant galactan F	M50	34	Nil	Pleurisy* +ve	5/7
	S73	34	Nil	Nil	3/7
	W19	34	R. carpus* +ve	Nil	1/7
	W35	23	Nil	Dark red lotule (no cultures of lung) HB+‡	8/8
No galactan (controls)	M47	23	Nil	Collapse (no cultures of lung)	4/8
	S84	34	Nil	HB+‡	2/8
	W34	34	Possibly lame	Nil	1/8
	W49	34	Nil		0/8

* Culture from lesion negative or positive for *M. mycoides*.

† LDL = Left diaphragmatic lobe; RDL = right diaphragmatic lobe.

‡ HB = Heart blood culture.

§ Number of days on which cultures were positive over the number of days on which samples were taken.

of a filamentous culture of the V5 strain of *M. mycoides* and were 17, 17 and 13 mth old respectively. This suggested that injection of galactan into infected older animals might cause pleurisy.

To test this hypothesis, four cattle, the youngest of which was 30 mth old, were given intravenous injections of galactan at a total dose rate of 2 mg per kg and a subcutaneous injection of 1 ml of filamentous culture of the V5 strain. Galactan F from Gladysdale, V5 and KH3J strains was pooled in this experiment. The cattle were killed 27 or 28 days after the injection of *M. mycoides*.

One animal developed pleurisy from which *M. mycoides* was isolated. One

had a lesion in the lung, which gave a positive culture and although not sufficiently characteristic to be pathognomic was consistent in appearance with a CBPP lesion. Lesions were present in the joints of one animal and in a bursa of another. Only one had a high proportion of positive blood cultures.

A second and larger experiment was then carried out. Six adult cattle, aged at least 44 mth, were given injections of galactan F and a filamentous culture of the V5 strain, and another six were given culture alone.

At necropsy none of the galactan-treated animals had pleurisy, but five had small lung lesions from which *M. mycoides* was isolated. In three of the five the organism was recovered only from the lesions and not from the heart blood. The lesions could have been due to specific infection, although the one lesion examined histologically was not typical of CBPP. In the other two cattle, organisms were recovered from the lesions and the heart blood; those recovered from the lesions were probably in the bloodstream. However, one of these lesions was similar to a sequestrum when examined histologically. Four of the six animals had joint lesions and three had a high proportion of positive blood cultures.

Four of the six control cattle were killed and necropsies carried out; the other two showed no signs of localisation of infection in the joints or the thoracic cavity and were retained for other experiments. None of the four had pleurisy and, though one had lung lesions from which *M. mycoides* was isolated, organisms were also present in heart blood cultures. None had joint lesions nor a high proportion of positive blood cultures.

The results of these experiments were not conclusive and a third further experiment with six animals treated with galactan F, and six untreated controls, was carried out. The method and the ages of the cattle were similar to those of the second experiment except that all lesions in the lung, whether suggestive of CBPP or not, were submitted for culture for both mycoplasmas and other organisms and for histological examination. The results are given in table III.

Four of the six galactan-treated animals had lung lesions from which *M. mycoides* was recovered, but all six had positive heart-blood cultures. Although one was complicated by the presence of parasites, the histological appearance of three lesions was consistent with that of CBPP; *M. mycoides* was isolated from two of these. None of the lesions was more than 5 cm³ in volume. Five of the six had joint lesions and all six had a high proportion of positive blood cultures.

Three of the four controls that were examined at necropsy had lung lesions from which *M. mycoides* was isolated and one of these did not have a positive heart-blood culture. The histology of two of these was consistent with that of CBPP, but only one was positive for *M. mycoides* on culture. Both lesions were small as in the case of those in the galactan-treated cattle.

In this last experiment severe kidney lesions were seen in three of the six cattle treated with galactan. They appeared as pale, depressed, often haemorrhagic areas up to 2 cm in diameter on the outer surface of the kidney. The lesion extended through the cortex into the medulla to produce a pale, wedge-shaped area.

TABLE III

Effect of circulating galactan F on the localisation of the V5 strain of M. mycoides in the pleural cavity and lungs of adult cattle after subcutaneous inoculation of a V5 challenge dose

Group given	Animal no.	Period to slaughter (days)	Joint lesions	Lung lesions			Blood cultures positive‡
				Site*	<i>M. mycoides</i> † culture	Histology	
Galactan F	N24	22	L. and R. tarsus	LDL RDL	+(HB+) +	CBPP§	6/6
	N29	28	L. and R. tarsus L. and R. femorotibial	RAL R. access RCL	— —(HB+) —		
	N30	14	L. acetabular R. tarsus	RCL	—(HB+)	...	8/8
	N34	29	None	LDL LDL ventral LDL posterior RDL RDL ventral RDL posterior	— —(HB+) — — + —	CBPP§	4/4
	N35	26	L. acetabular L. and R. humero-scapular	LAL RAL	++ (HB+) +	...	
	N37	29	L. carpus L. humero-radial L. metacarpophalangeal R. acetabular	LDL RAL RAL dorsal	—(HB+) — +	early CBPP§	8/8
	N25	35	None	LCL LDL ventral LDL posterior RCL	—(HB—) — + —	...	6/10
	N26	33	None	RDL	—(HB—)	early CBPP§	4/10
No galactan	N28	26	None	LDL RAL RCL RDL	—(HB+) + + —	...	6/7
	N36	21	None	LCL LDL anterior LDL posterior RAL RCL	+(HB+) + + + +	CBPP§	3/6

* LAL = Left apical lobe; LCL = left cardiac lobe; LDL = left diaphragmatic lobe; RAL = right apical lobe; R. access. = right accessory lobe; RCL = right cardiac lobe; RDL = right diaphragmatic lobe.

† + = *M. mycoides* isolated from given site; HB refers to heart blood cultures.

‡ Number of days on which cultures were positive/number of days on which samples were taken.

§ None of these lesions exceeded 5 cm³ in volume.

|| An α -haemolytic streptococcus was also isolated.

Histologically there was extensive damage to the cortex; interstitial tissue was infiltrated with mononuclear cells, tubules were distended and sometimes contained casts. In some cases the tubular epithelium was degenerating and

TABLE IV

Response after "natural exposure" to CBPP of cattle treated with W fraction of the cellular galactan of M. mycoides in Freund's adjuvant

Group	Animal no.	Period to slaughter (days)	Time from challenge to clinical response (days)	Time from challenge to rise in CF titre (days)	Necropsy findings	Scores*
Given W fraction in Freund's adjuvant	A58	109	27	36	CBPP and <i>Corynebacterium pyogenes</i>	13
	H88	30	20	...†	typical acute CBPP	15
	H95	32	19	...	typical acute CBPP	15
	J8	83	...	40	no lesions	3
	J10	53	36	43	typical acute CBPP	18
	J11	41	29	33	typical acute CBPP	18
	M52	101	...	73	no lesions	1
	M85	92	...	40	no lesions	1
Untreated (susceptible controls)	H2	37	19	20	typical acute CBPP	14
	H9	28	19	19	typical acute CBPP	13
	Z2	90	...	47	no lesions	2
	Z10	70	26	26	moderate chronic CBPP	13
	Z13	56	...	15	moderate chronic CBPP	11
	Z16	83	...	29	no lesions	3
	Z17	69	24	19	moderate chronic CBPP	9
	Z26	36	16	16	typical acute CBPP	18

* Mean score of cattle treated with W fraction in Freund's adjuvant 10.50 ± 2.59 ; mean score of control cattle 10.375 ± 1.945 (see *Methods*).

† ... = No response.

glomeruli were shrunken or degenerating and surrounded by dense connective tissue. Lesions of this type were not present in the kidneys of cattle that did not receive galactan.

Effect of previous injection of the W fraction of galactan from M. mycoides on the susceptibility of cattle to lung infection with M. mycoides

Eight cattle were each given two intramuscular injections 2 wk apart, first with 2.7 mg of W fraction in 5.5 ml of saline and then 1.0 mg of W fraction in 2.0 ml of saline mixed with Freund's adjuvant in the proportion 1 of W solution

to 2 of adjuvant. Complement-fixing (CF) antibody appeared in seven of the eight after the second injection and in three cattle reached a titre of 1 in 40. W fixes complement in the presence of cholesterol, lecithin and antibody (Buttery, unpublished) so it is assumed that complement-fixing antibodies to W were being measured.

Four of these animals were placed in a "natural exposure" experiment (Hudson and Turner) at 19 days and four at 42 days after the last injection of fraction W. Their susceptibility to infection was compared with that of eight cattle randomly selected from untreated control animals in the same experiment. The responses of the two groups are shown in table IV.

TABLE V

Effect of circulating galactan F on the clearance of M. mycoides from the bloodstream after intravenous inoculation of culture in cattle

Group	Animal no.	Weight (kg)	Number of organisms (10^{11})	Number of organisms per ml in blood at				
				$\frac{1}{2}$ hr	1 hr	3 hr	24 hr	48 hr
Given galactan F	W24	180	0.36	10^2	10^3	10^2	10	1
	A80	208	1.68	10^4	10^4	$>10^5$	$>10^4$	...*
	S72	312	1.69	1	1	1	0.25	10
	S98	249	1.36	10^2	10^3	10^3	10	10
Given no galactan (controls)	A79	229	1.68	10	1	10^3	1	0.25
	A82	172	0.71	10	10^2	10^2	1	10
	S81	239	1.31	0	0	0	0.25	0
	S63	316	1.71	0.25	1	10^2	1	10

* ... = No data.

There was little difference in the proportion of animals in each group that became infected. The severity of the disease may have been greater in the group treated with fraction W; the scores were higher and four animals died or were killed *in extremis*, as against three in the control group, but these differences were not significant.

There was, however, a marked difference in the two groups of cattle in the time-interval between the advent of complement-fixing antibody and the appearance of clinical signs. In the group treated with fraction W, three animals developed CF antibody from 4 to 9 days after the first temperature rise and two animals died without showing any CF response at all. In the normal cattle, as is usual in such experiments, the CF response occurred before or at the same time as the clinical response.

Effect of galactan F on the clearance of M. mycoides injected intravenously into cattle

Galactan F was injected intravenously into four cattle in a single dose of 1 mg per kg. Two hours later, a culture of the V5 vaccine strain of *M. mycoides*

was injected *intravenously* at the rate of 0.2 ml (approximately 4×10^8 organisms) per kg body weight. Four untreated control cattle were given an injection of a similar dose of organisms. Blood samples were taken at 0.5, 1, 3, 24 and 48 hr and titrated for *M. mycoides* by the method of Miles and Misra (Miles *et al.*). The detailed results are set out in table V.

In three of the four galactan-treated cattle, organisms were present in the blood in moderately high numbers at 0.5, 1 and 3 hr, but there were fewer at 24 hr and 48 hr. The fourth animal showed a low count throughout the entire sampling period. The untreated control animals had fewer circulating organisms at 0.5 and 1 hr, but in three of the four the number increased to a maximum at 3 hr and then declined. It was concluded, after statistical analyses, that galactan F did not inhibit clearance from the bloodstream.

Acute reaction to the galactan

In this series of experiments, 53 cattle received intravenous injections of galactan; three died, five were severely affected and went down, ten were severely affected but remained standing, 17 were mildly affected and 18 did not show a reaction. Signs of the reaction, described by Hudson *et al.*, were seen within 30 s of commencement of the injection and with as little as 20 mg of galactan.

At necropsy, dead cattle showed marked congestion of trachea, bronchi, lungs, jejunum, ileum and small colon and subendocardial haemorrhages in the left ventricle. On histological examination the congestion was confirmed and there was a small amount of haemorrhage and oedema in the lung alveoli.

DISCUSSION

These experiments confirm the findings of Hudson *et al.* (1967) that cattle treated with galactan from *M. mycoides* and given subcutaneous injections of a culture of *M. mycoides* develop a high incidence of joint lesions and a marked mycoplasmaemia. In addition, two other changes were observed in these later experiments; pleurisy occurred in four of the 36 cattle treated with an adequate dose of the active fraction of galactan, and kidney lesions, probably infarcts, occurred in three out of six animals in the one experiment where kidneys could be adequately examined early in the disease.

Small lesions were seen in the lungs of cattle that had received galactan and subcutaneous injections of *M. mycoides*, but these also occurred in cattle that received *M. mycoides* alone, so they could not be attributed to the effects of the galactan. This contrasts with the experiments of Hudson and Leaver (1965), where no lung lesions were found in, nor were organisms isolated from, the lungs of 80 cattle given inoculations at tail-tip, 40 with smaller and 40 with much larger doses of the same V5 strain of *M. mycoides*, and killed after an interval of 4–5 wk.

The significance of these lesions, which were only 1–5 cm³ in volume, is unknown. They are common in cattle with pleurisy caused by injecting *M. mycoides* subcutaneously, but they do not progress to lesions comparable

in size with those seen in CBPP. They involve only a few lobules and show exudative changes in alveoli, thrombosis of lymphatics and blood vessels and inflammation of bronchioles, all of which occur in CBPP. Indeed, the only criteria on which they can be separated from CBPP lesions is their small size and their position on or just beneath the surface of the lung.

The failure of these lesions to extend might be due to their location. It has been suggested that the valves in the lymphatic system draining the peripheral part of the lung are arranged so as to prevent the flow of lymph through the lung to the hilus (Miller, 1947). Lymphatics are involved in CBPP lesions (Meyer, 1909; Orue, Mémerly and Thiéry, 1961; Provost, 1969; Lloyd and Trethewie, 1970) and if there is no centripetal flow from this superficial zone then the small lesions there will not increase in size. Although this view of lymphatic drainage has been challenged (Simer, 1952), the behaviour of *M. mycoides* infections in the pleural cavity of cattle suggests that it is true for this species (Lloyd and Trethewie).

One substance does provoke lung lesions in cattle challenged with *M. mycoides*, namely fluid from sterile embryonated egg. If this material is injected together with V5 vaccine it induces lesions, sometimes large, that are indistinguishable from those of CBPP (Hudson, 1965; Hudson and Leaver). The small lesions observed in the present series of experiments are not comparable with the egg-induced lesions.

The joint lesions were similar to those that occur in calves after vaccination with moderately virulent strains of *M. mycoides* (Turner and Trethewie, 1961). The part played by the galactan in their pathogenesis is obscure; it might have localised in joints, reacted with antibody produced by the infection with *M. mycoides*, and so caused lesions. Alternatively, the organisms in the joints might have been responsible for the lesions, the galactan suppressing the defensive response to the infection. Joint localisation occurring after enhancement of a mycoplasma infection in rats by a polysaccharide has been reported by Delbarre *et al.* (1964), who showed that *M. arthritidis* produced arthritis more readily if the organism was injected in a solution of dextran. These workers suggested that the dextran caused local oedema at the site of injection and enhanced the infection. The dextran might have acted systemically in the same way as galactan F in *M. mycoides* infections.

Hudson *et al.* found that cattle treated with galactan F from the V5 strain and then with a subcutaneous injection of a culture of the KH3J strain developed mycoplasmaemia whereas those that were given an injection of KH3J alone did not. Their work and the experiments reported here have shown that the subcutaneous inoculation of V5 strain alone will produce mycoplasmaemia. We have shown that the galactan F from Gladysdale and KH3J strains has similar effects at the same concentration. This suggests that the KH3J strain may produce less galactan than the Gladysdale strain. Gourlay and Thrower, and Buttery (unpublished) have suggested that the quantity of galactan produced by a strain of *M. mycoides* might vary with its virulence.

Mycoplasmaemia did not occur in immune animals treated with galactan and challenged with *M. mycoides*. This suggests that the polysaccharide does

not depress immunity, but a dose rate higher than the 2 mg per kg may be required to demonstrate this. Failure to demonstrate depression of blood-stream-clearing mechanisms suggests that the persistence of organisms in these experiments was not due to depression of that function of the reticulo-endothelial system by the galactan.

These studies have provided other information about the galactan. A total dose of at least 0.66 mg per kg is required to induce joint lesions and mycoplasmaemia in animals infected subcutaneously with *M. mycoides* and the fraction of the galactan that is responsible for these activities is the one released into the medium during the growth of the organism in culture. There is no qualitative difference in the activity of galactan derived from virulent and non-virulent strains.

The W fraction of the galactan had not previously been tested for its ability to induce resistance; the other fraction (galactan F) did not induce immunity (Hudson *et al.*). The present experiments indicate that W does not contain a protective antigen and that it might have increased the susceptibility to infection. It reduced the CF antibody response to infection; two animals died without showing a CF response and in three others the response was much later than normal. This suggests that it may reduce responsiveness to the antigen(s) responsible for CF antibody production.

Kidney lesions similar to those reported here are common in cattle with CBPP, but are rarely seen in cattle with experimental subcutaneous *M. mycoides* infections in this laboratory. They have been observed by one of us (J. R. H.) in cattle dying in the field as a result of vaccination accidents; the infection caused by the vaccine in these circumstances was much more severe than usual. It is not known why the incidence of kidney lesions in cattle with subcutaneous *M. mycoides* infections is different from that in cattle that have CBPP, severe vaccination responses or subcutaneous *M. mycoides* infections with concurrent galactan treatment.

The acute effects of the galactan were similar to those observed by Hudson *et al.* No explanation can be offered for this effect, but further investigations are being undertaken.

SUMMARY

The injection of galactan extracted from *Mycoplasma mycoides* into cattle subsequently infected with *M. mycoides* induced lesions in joints and kidneys and a prolonged mycoplasmaemia. Several treated animals developed pleurisy, but the only lung lesions were small and were present in both galactan-treated and control animals. These effects occurred with galactan prepared from both virulent and avirulent strains and were evident at a dose rate of 0.66 mg per kg but not at 0.22 mg per kg. The supernatant fraction galactan F and the cell fraction L, which might be the same as the supernatant fraction, were equally active. The other cell fraction W did not induce lesions nor mycoplasmaemia.

The galactan had no effect on the rate of clearance of *M. mycoides* injected intravenously into cattle, nor did it reduce the resistance of vaccinated cattle.

Although the W fraction, when injected with Freund's adjuvant into cattle, provoked CF antibody production, it did not induce resistance to airborne CBPP infection. It suppressed or delayed the complement-fixing antibody response to *M. mycoides* in those cattle that became infected after exposure to airborne infection.

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