THE *IN VITRO* ACTION OF DISINFECTANTS AND THE APPLICATION OF CTAB IN THE CONTROL OF *STR. AGALACTIAE* MASTITIS

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(With 1 Figure in the Text)

Recent work (I.B.A.H. Review Series, 1944) indicates that *Str. agalactiae* may be located on the surface of the udder, on dairy utensils and on the hands of the personnel engaged in milking. These sites become contaminated from infected milk during the milking process, and it is generally recognized that the use of a non-irritant disinfectant capable of destroying the organism in these sites would be a useful adjunct to other methods of controlling the spread of chronic mastitis. The choice of disinfectant for practical usage would be determined by its maximal *in vitro* potency, its rapidity and persistence of destructive action and its non-irritant effect on living tissues when applied regularly.

The experiments reported in this paper compare the potency of several disinfectants which might be employed for this purpose, and while in Part I of the paper it will be shown that CTAB was the most potent of the disinfectants tested, Part II shows that when persistently applied to cows' teats, CTAB has an irritant effect which makes it unsuitable for regular use in practice.

PART I. DISINFECTANTS USED

The action on *Str. agalactiae* of the following disinfectants was investigated:

(*a*) *Those containing chlorine as the active principle*. Disinfectants of this group, on account of their relative cheapness, are commonly used in dairy practice. Chloros contains 10 % by weight of available chlorine, while Deosan and Milton are disinfectants of the same type and are probably of the same chemical composition as Chloros; the phenol coefficient of these disinfectants is stated to be 80.

(*b*) *Chloroxylenol solutions*. Two preparations, namely, Dettol and Supersan, were tested. The latter preparation is said to have a phenol coefficient of 6 and contains 5 % chloroxylenol.

(*c*) *CTAB* (*cetyl-trimethyl-ammonium bromide*). The use of this compound was first suggested in 1942 by Barnes, who also described its detergent and disinfectant properties. The crude substance is a brown, greasy powder, soluble in water, and is dispensed as a 7·5 % solution under the trade name of 'Cetavalon'.

Technique employed for comparing the *in vitro* potency of disinfectants

The technique involved in comparing the various disinfectants was to test their efficiency at varying dilutions against calculated numbers of *Str. agalactiae* at room temperature (18° C.). At intervals one drop (0·02 ml.) of each concentration was removed, as in the Rideal-Walker test, but instead of subculturing into broth, the number of viable organisms in the drop was determined by roll-tube counts (Wilson, 1922), using blood agar as the culture medium. The test was carried out in duplicate.

This method of evaluating the 'end-point' of each disinfectant was chosen because a better appreciation could be made of the death-rate by a quantitative method than by subculturing in broth.

In deciding the time during which the organism was in contact with the disinfectant dilution, a maximum period of 4 min. was allowed, since in routine dairy practice it would not be convenient to permit a longer time for contact with hands, utensils, etc. In quoting effective concentrations of disinfectants, certain authors have given results based on the reaction between the test organism and disinfectant after 18 hr. at 37° C., an interval which would not be applicable in practice. The medium used for conducting the test and making the dilutions of disinfectant was sterile tap water.

Results

The results of a preliminary test are shown in Table 1.

It may be seen that in the case of Chloros, Milton, Deosan and CTAB, no end-point was observed in the range of dilutions chosen, since all were bactericidal. An interesting comparison, however, may be made between the last three substances. After

Table 1

Disinfectant	Dilution	No. of organisms in 0·02 ml. after contact for 30 sec.	1 min.	2 min.	4 min.
Chloros, Milton, Deosan, CTAB 7·5 %	1/100–1/1000	0	0	0	0
Phenol	**1/100**	20	0	0	0
	1/200 and above	700	700	700	700
Dettol	1/100	0	0	0	0
	1/200	3	0	0	0
	1/500	39	4	3	2
	1/1000	305	100	36	22
Supersan	1/100–1/200	0	0	0	0
	1/500	15	0	0	0
	1/1000	700	700	700	700
Control	Tap water				700

0 = no growth.

contact for 1 min., phenol at 1/100 proved effective, Dettol at 1/200 and Supersan at 1/500; these results agree closely with their accepted phenol coefficients.

In a further experiment shown in Table 2, where lower concentrations of chlorine disinfectants and of CTAB were compared, one drop of culture dilution was removed into a roll tube after contact for 30 sec. only, since it had been found that the end-point for a given concentration of disinfectant was usually in no way different at 30 sec. from that at later intervals. If the organisms survived for the shorter period, they would do so for at least 4 min. It appeared, therefore, that the deciding factor in determining the death-rate of *Str. agalactiae* under the conditions of these experiments was the concentration of the disinfectant present rather than the time of contact. Furthermore, progressive dilutions of disinfectant gave fairly sharp end-points at which the effective concentration could be decided.

Table 2

Dilution	1/9000	1/27,000	1/81,000	1/243,000	Control
	No. of organisms in 0·02 ml. after contact for 30 sec. in tap water				
Chloros	0	0	63	504	504
Milton	0	528	500	500	504
Deosan	0	500	500	500	504
CTAB 7·5 %	0	0	0	500	504

Table 2 shows that the end-points of the chlorine disinfectants are approximately the same and that Milton and Deosan have a phenol coefficient of about 90 (a figure which agrees closely with that claimed by the makers), while the value for Chloros is even higher.

According to this test, moreover, the superiority of CTAB is shown, since a dilution of 1/81,000 of 7·5 % crude CTAB solution was effective. Hence the calculated phenol coefficient of *crude* undiluted CTAB is about 105,300.

The in vitro *action of CTAB on* Str. agalactiae *in broth*

A further experiment to test the *in vitro* activity of CTAB after prolonged contact with *Str. agalactiae* in a broth medium was carried out. Progressive dilutions of CTAB were made in Hartley broth, as shown in Table 3.

Various strains of *Str. agalactiae* were grown for 18 hr. in Hartley broth. The broths were centrifuged and the deposit suspended in Ringer's solution, which was matched to the opacity of Brown's tube 2. Each dilution of CTAB received 0·02 ml. of this standard suspension. The same inoculum was placed in control broths containing no CTAB, while CTAB broths which were not inoculated were also incubated to check the sterility of the solution.

The tests were read after 18 and again after 36 hr. incubation at 37° C. The end-point for each strain was taken to be the last tube in which growth was inhibited, and absence of growth was assessed by naked-eye appearance.

Results after 36 hr. incubation are shown in Table 3.

It may be seen from Table 3 that while there is variation in the effective lethal concentrations with the different strains, the majority, i.e. 11 out of 14, were destroyed at CTAB dilutions of 1/40,000–1/80,000. Thus it appears that there is a close agreement in the results of these two methods of testing, since the end-point at 30 sec. and at 36 hr. are nearly the same. It may be concluded, therefore, that the activity of CTAB against *Str. agalactiae, in vitro*, is maximal within a period of 30 sec., and that where dilutions are not effective in this time, the organism

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can resist the action of disinfectant for a prolonged period.

Effect of organic matter on disinfectants

Under practical usage it is unlikely that disinfectants would, in fact, be acting on *Str. agalactiae* in such favourable conditions as plain water or broth, the test mediums previously employed, and their activity is likely to be affected by the presence of organic matter.

The fact that the potency of CTAB is reduced by organic matter has also been noted by Williams, Clayton-Cooper, Duncan & Miles (1943), who observed that the presence of 10% horse serum caused a 27-fold drop in its effective concentration.

CTAB as a skin disinfectant

It has been shown by Harrison (1941) and other workers (I.B.A.H. Review Series, 1944) that *Str. agalactiae* may be isolated from milkers' hands,

Table 3

Strain	Broth control	Dilutions of CTAB 7·5 %: 1/5000	1/10,000	1/20,000	1/40,000	1/80,000	1/160,000	1/320,000	1/640,000	1/1,280,000	1/2,560,000
Barnsley 1	+	0	0	0	0	0	0	+	+	+	+
Barnsley 2	+	0	0	0	0	0	+	+	+	+	+
Middlethwaite A	+	0	0	0	0	0	+	+	+	+	+
Middlethwaite B	+	0	0	0	+	+	+	+	+	+	+
Ferguson 6	+	0	0	0	0	0	+	+	+	+	+
S 84	+	0	0	0	0	0	+	+	+	+	+
S106	+	0	0	0	0	0	+	+	+	+	+
S107	+	0	0	0	0	+	+	+	+	+	+
S119	+	0	0	0	0	0	+	+	+	+	+
S121	+	0	0	0	0	+	+	+	+	+	+
N 49	+	0	0	0	0	0	0	+	+	+	+
FM 4/1	+	0	0	0	0	0	+	+	+	+	+
FM 6/2	+	0	0	0	0	+	+	+	+	+	+
S127	+	0	0	0	0	+	+	+	+	+	+
Uninoculated CTAB broth control		0	0	0	0	0	0	0	0	0	0

+ = growth. 0 = no growth.

Table 4

Disinfectant	Medium	Dilutions: 1/800	1/1000	1/2000	1/3000	1/9000	1/27,000	1/81,000	Control
		No. of organisms in 0·02 ml. after contact for 30 sec.							
Chloros	Tap water	0	0	0	0	0	0	25	252
	Tap water + 10 % milk	0	283	297	294	261	256	268	
CTAB 7·5 %	Tap water	0	0	0	0	0	0	7	258
	Tap water + 10 % milk	0	104	96	187	206	209	207	

It is difficult to estimate the power of disinfectants under all such adverse conditions, but in a further series of experiments, comparison was made of the efficiency of Chloros and of CTAB when acting on *Str. agalactiae* in sterile tap water alone as well as in water containing 10 % sterile milk.

The results shown in Table 4 confirmed the efficiency of Chloros and CTAB against *Str. agalactiae* exposed in water, but when 10 % milk was included, their minimal effective concentration was reduced to 1/800.

which may be a frequent cause of the spread of mastitis. Attention may also be drawn to the findings of the latter workers on the frequency with which the organism may be recovered from the skin of cows' teats.

Since the superiority of CTAB over other disinfectants was shown by preliminary *in vitro* tests, a further series of experiments was conducted with a view to demonstrating the duration of its disinfectant action when applied to milkers' hands and cows' teats.

Table 5

Strain	Dilution of strain	Time after application of CTAB: 1 hr. Test pastille	1 hr. Control pastille	3 hr. Test pastille	3 hr. Control pastille	6 hr. Test pastille	6 hr. Control pastille
Aconite	10^{-1}	0	In.	0	In.	In.	In.
	10^{-2}	0	In.	0	In.	In.	In.
	10^{-3}	0	In.	0	In.	In.	In.
Mercy	10^{-1}	0	In.	0	In.	In.	In.
	10^{-2}	0	In.	0	In.	In.	In.
	10^{-3}	0	In.	0	In.	In.	In.

0 = no growth after 48 hr. at 37° C. In. = innumerable haemolytic colonies.

(1) *Action on the skin of hands and on cows' teats*

The method employed for demonstrating this effect was that described by Williams *et al.* (1943), which consisted in applying to the treated skin of the hand pastilles of blood agar containing *Str. agalactiae*. These pastilles were prepared by removal with a sterile cork borer of small cylinders from poured agar plates inoculated with varying numbers of *Str. agalactiae*. The pastilles measured 1 cm. in diameter and 2 mm. in thickness. After 5 min. application to the palm of the hand, the pastilles were removed and incubated for 48 hr. on the surface of plain agar. Pastilles were also applied to untreated skin in order to rule out the existence of the lethal factor for streptococci which normally occurs in the skin of certain individuals (Burtenshaw, 1942).

Two strains of *Str. agalactiae* grown in Hartley broth for 18 hr. were diluted 10^{-1}, 10^{-2} and 10^{-3} and plated in deep blood agar. Pastilles from agars containing these dilutions were then applied to the skin of the hands. Previous to testing, the hands were washed with soap and water and rinsed without drying. Then 1 % CTAB solution was rubbed vigorously on them for 5 min., by which time the hands were dry. The hands were sampled by the pastille technique 1, 3 and 6 hr. after the application of the CTAB. Results are shown in Table 5.

It may be seen in Table 5 that the disinfectant action of CTAB persists on the dry hand for at least 3 hr.

The disinfectant action of CTAB on the teats of cows was tested in a similar manner except that it was applied in the form of a cream, containing 1 % concentration, which will be described later. Two cows were employed in this experiment. *Str. agalactiae* was not found on the teats of either of these animals when the teats were swabbed by the method described in I.B.A.H. Review Series (1944). The pastilles were applied to the teats for 5 min. at varying intervals after the teats had been liberally dressed with 1 % CTAB cream.

Table 6. *Cow* 1

Time	Dilutions of culture: 10^{-1}	10^{-2}	10^{-3}
Before CTAB	In.	In.	In.
30 min. after CTAB	0	0	0
1 hr. after CTAB	0	0	0
3 hr. after CTAB	0	< 10	0
4 hr. after CTAB	Complete lysis at	3	3
5 hr. after CTAB	edge of pastille	0	0

Table 7. *Cow* 2

Time	CTAB-treated teats, Dilutions of culture: 10^{-1}	10^{-2}	10^{-3}	Untreated teats, Dilutions of culture: 10^{-1}	10^{-2}	10^{-3}
Before CTAB	...	...	...	In.	In.	In.
3 hr. after CTAB	0	0	...	In.	In.	...
5 hr. after CTAB	0	< 10	0	In.	...	...
6½ hr. after CTAB	< 10	0	< 10	In.	In.	In.
7½ hr. after CTAB	In.	Some growth	< 10	In.	...	...
8½ hr. after CTAB	< 10	0	0	In.	In.	In.
9½ hr. after CTAB	In.	< 10	0	...	...	...

... = no observation.

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In cow 2 the left-side teats were treated with CTAB and the right-side teats were untreated. Pastilles were applied to all teats, the right teats acting as controls.

In these tests it was difficult to ensure that the pastille was in complete apposition with the teat throughout the 5 min. allowed for contact. Movements of the cow tended to cause a lifting of the pastille. This difficulty may explain the irregular patches of growth in some pastilles, when other pastilles applied at the same time failed to show growth. Whatever the explanation may be, it appeared that the activity of 1 % CTAB cream persisted for at least 3 hr. on cows' teats in sufficient concentration to inhibit growth in pastilles applied for 5 min., and some inhibition could be demonstrated at 8½ hr.

(2) *Action in the presence of milk.*

A further experiment was carried out to ascertain the effect of milk on the action of CTAB when applied to the hands. The same technique was employed as in the earlier experiment, and the pastille method was used to find the persistence of the disinfectant.

The hands were washed with 1 % CTAB solution and tested with pastilles 1 hr. later. At this time 2 ml. of sterilized milk were rubbed into the hands in order to provide a coating of milk in the same manner as might occur in the practice of milking. At intervals of 30 min. and 2 hr. after the application of milk, further tests were carried out. The results of this experiment are shown in Table 8.

Table 8

Dilution of strain	Before CTAB		1 hr. after CTAB treatment		After milk			
					30 min.		2 hr.	
	Test pastille	Control pastille	Test	Control	Test	Control	Test	Control
10^{-1}	In.	In.	0	In.	0	In.	In.	In.
10^{-2}	In.	In.	0	In.	0	In.	In.	In.
10^{-3}	In.	In.	6	In.	50	In.	In.	In.

It will be seen that the disinfectant effect of 1 % CTAB had disappeared 2 hr. after the application of milk, i.e. 3 hr. after treatment with CTAB, whereas in the absence of milk it was still fully effective at 3 hr. (see Table 5).

CTAB in a vanishing cream

In order to obviate the possible irritant properties of CTAB in a watery solution on repeated application, Williams *et al.* (1943) suggested the desirability of adding oils, and for this purpose prepared a vanishing cream. It was thought that the application of such a cream would possess an advantage over the commonly employed dairy routine disinfection in that it could be used in one operation, i.e. immediately before stripping each cow, for disinfecting not only the milkers' hands but also the teats.

The cream described by Williams *et al.* contained 1 % CTAB in a 10 % arachis oil and 5 % lanette wax base. On testing the bactericidal activity of such a preparation, they found that although the dispersed oil and fat deviated the power of CTAB to some extent, 1 % CTAB in the full cream had considerable activity when tested by the cup-plate technique of Rose and Miller (1939).

The following test was carried out with a cream containing 1 % crude CTAB, 4·8 % arachis oil and 2 % lanette wax, which, after homogenizing, gave a suitable emulsion. Comparison was made with the inhibition zone produced by this cream and with that of a 1 % aqueous solution of CTAB. The method of preparing the plates was similar to that described by Rose & Miller, except that the diameter of the cup containing disinfectant was 12 mm. instead of 15 mm., and that *Str. agalactiae* was used as the test organism.

Varying dilutions of 18 hr. Hartley broth cultures were inoculated into Petri dishes and mixed with blood agar. When the agar was set, a plug of agar 12 mm. in diameter was removed with a sterile cork borer. The bottom of the cup was sealed with 2 or

Table 9. *Comparison of inhibitory effect of CTAB in 1 % aqueous solution and in a vanishing cream at the same strength*

Strain	Dilution of strain	Diameter in mm. of area of inhibition: In aqueous solution	In cream
8	10^{-3}	3	2
	10^{-4}	3	3
	10^{-5}	3	4
16	10^{-3}	3	2
	10^{-4}	3	3
	10^{-5}	3	5
20	10^{-3}	3	2
	10^{-4}	3	3
	10^{-5}	3	4
S107	10^{-3}	7	6
	10^{-4}	5	6
	10^{-5}	6	6

3 drops of plain agar and the cup filled with the preparation of CTAB under test. Control plates had distilled water in the cups. Measurements of the zone of inhibition were made after 48 hr. incubation at 37° C.

It may be seen that the inclusion of CTAB in the vanishing cream has only slightly reduced the distance to which it can diffuse in the agar medium, when compared with its activity dissolved in water. It is also evident that this applies irrespective of the number of organisms in the plate. It was expected, therefore, that such a cream would, in practice, be no less effective than a watery solution in destroying *Str. agalactiae* on the surface of milkers' hands and cows' udders.

Summary

1. *In vitro* experiments carried out by a modified Rideal-Walker technique showed the superiority of CTAB over chlorine and chloroxylenol disinfectants against *Str. agalactiae*.

2. The disinfectant action of 1% CTAB persisted on the surface of dry hands and cows' teats for a period of at least 3 hr. Subsequent moistening of the hands with milk reduced the efficiency of this disinfectant.

3. For the prevention of dissemination of *Str. agalactiae* by means of milkers' hands and at the same time to reduce the incidence of infection on the surface of teats, a lanette wax-oil base containing 1% CTAB was tested. It was found that the inclusion in the cream base of CTAB did not reduce its bactericidal efficiency.

PART II. AN ATTEMPT TO CONTROL THE SPREAD OF *STR. AGALACTIAE* INFECTION IN A DAIRY HERD BY MEANS OF CTAB

The evidence presented in Part I showed that of the disinfectants tested, CTAB (cetyl-trimethyl-ammonium bromide) was the most effective in destroying *Str. agalactiae* under the conditions of test. The experiments recorded in Part I suggested that CTAB might prove useful under routine dairy conditions. A field trial was, therefore, undertaken in one of the herds at this Field Station. This herd had been under close bacteriological supervision since June 1941. The incidence of *Str. agalactiae* on the outside of the teats and in the milk had been closely followed, and detailed results are given in I.B.A.H. Review Series (1944) under the heading "Herd C2". The technique of sampling, which is fully described in the same Bulletin, may be summarized as follows: Teat swabs were inoculated into sterile milk and incubated overnight, and composite milk samples from all four quarters were incubated for 48 hr. Teat and milk samples, after this preliminary incubation, were sown on the surface of Edwards' crystal-violet aesculin blood agar medium to which had been added 1/2000 thallium acetate. Final readings of the plates were made after 48 hr. incubation. Colonies were identified by precipitin tests with group B *Streptococcus* anti-serum.

The evidence in Part I showed that the potency of CTAB incorporated in an oily base was not seriously reduced when compared with a watery solution, and for reasons already stated, it was thought that the application of such an antiseptic cream might have certain advantages for regular use at milking when applied to skin surfaces. A 1% solution of crude CTAB was incorporated in a base composed of 4·8% arachis oil and 2% lanette wax and emulsified in a homogenizer. The resultant preparation was a cream with a consistency similar to that of a toilet skin preparation.

Methods employed at the time of milking

The procedure adopted in the dairy was as follows. The cleansing of the udders was carried out with sterile cloths using separate cloths for each cow. The udder cloths were soaked with clean water drawn from a single container provided with a tap. The use of fresh water supplied in this way avoided contamination of the washing water by the attendant's hands, which occurs when the cloths are immersed in buckets. Contact between the udder and the washer's hands was avoided by keeping the cloth over the hands. No disinfectant was used for udder washing.

The cream was issued to the dairy personnel in small containers which they carried with them during milking. One milker undertook the removal of foremilk from all the cows into a strip cup, and he anointed his hands liberally with cream between each cow. The cows were then milked by machine. Before the teat cups were placed on a cow they were plunged up and down three or four times in a bucket of clean water to remove residual milk, and then washed in a similar manner in a bucket containing 8 oz. of Chloros in 2 gal. of water. The contents of the buckets were changed after every fifteen cows. The technique described had been in daily use for some months previous to the present experiment, in which milkers employed in stripping covered their hands with 1% CTAB cream. Before they left the cow the same cream was also liberally applied to the teats of the animal. Fresh applications of 1% CTAB cream were applied to the milkers' hands between each cow.

Results

The results are summarized in Table 10 and Fig. 1.

In Table 10 the number of cows examined at each test is shown, and the results are separated to

Table 10. *Showing the incidence of* Str. agalactiae *infection in a herd before, during and after the use of CTAB as a disinfectant*

CTAB treatment commenced 10. viii. 43. *Amount of CTAB applied reduced 10. xi. 43.* CTAB treatment discontinued 15. xii. 43.

	Teats					
Date	No tested	No. positive	% positive	New positive	Cumulative no. positive	Cumulative % positive
29. iii. 43	51	24	47	—	24	44·4
27. v. 43	48	33	68·7	16	40	74·0
26. vi. 43	49	10	20·4	1	41	75·9
5. viii. 43	47	6	12·7	1	42	77·7
11. x. 43	52	2	3·8	—	2	3·7
21. x. 43	52	3	5·7	3	5	9·2
26. x. 43	52	1	1·9	0	5	9·2
2. xi. 43	53	2	3·7	1	6	11·1
9. xi. 43	52	4	7·6	3	9	16·6
23. xi. 43	53	9	16·9	5	14	25·9
7. xii. 43	52	23	44·2	14	28	51·8
11. i. 44	51	40	78·4	18	46	85·1
31. i. 44	53	40	75·4	3	49	90·7
8. ii. 44	53	37	69·8	1	50	92·5
28. ii. 44	54	39	72·2	0	50	92·5
15. iii. 44	54	29	53·7	1	51	94·4
4. iv. 44	54	22	40·7	0	51	94·4
27. iv. 44	35	19	54·2	1	52	96·2

	Milks					
Date	No. tested	No. positive	% positive	New positive	Cumulative positive	Cumulative % positive
29. iii. 43	50	17	34·0	—	30	55·5
27. v. 43	46	17	36·9	4	34	62·9
26. vi. 43	46	6	13·0	1	35	64·8
5. viii. 43	47	4	8·5	0	35	64·8
11. x. 43	46	6	13·0	—	6	11·1
21. x. 43	39	5	12·8	1	7	12·9
26. x. 43	43	7	16·2	2	9	16·6
2. xi. 43	40	7	17·5	2	11	20·3
9. xi. 43	38	6	15·7	2	13	24·0
23. xi. 43	33	9	27·2	2	15	27·7
7. xii. 43	37	13	35·1	5	20	37·0
11. i. 44	42	21	50	9	29	53·7
31. i. 44	43	25	58·1	8	37	68·5
8. ii. 44	47	23	48·9	2	39	72·2
28. ii. 44	42	27	64·2	3	42	77·7
15. iii. 44	40	24	60·0	3	45	83·3
4. iv. 44	38	25	65·7	2	47	87·0
27. iv. 44	37	16	43·2	0	47	87·0

Cows are only included in this table if they were teat and milk sampled in at least half the tests before and after CTAB treatment had commenced.

indicate the number and percentage of cows infected on the teat surface and in their milk at each test. In addition, a cumulative infection rate is shown for both sites and is obtained by adding each new infection found at one test to the preceding total infected cows. This figure has been further expressed as a percentage of the total number of animals from which milk and teat samples were examined on the same occasion in at least half of the total samplings before and after the application of CTAB. The actual number of such animals was fifty-four.

In Fig. 1 the cumulative rate expressed as a percentage is shown graphically and depicts the herd infection after 2 months of continuous twice-daily application of 1 % CTAB cream to the cows' teats and milkers' hands. From 11 October for approximately one month (five tests) the percentage of cows harbouring *Str. agalactiae* on the surface of the teat (teat rate) remained low (under 7·6 %) and constant. The same was true of the percentage of cows infected in their milk (milk rate), although the level of infection was higher.

At the end of this time, 10 November, it became obvious that a considerable number of cows had developed lesions of the teats, principally chaps and fissures, and the dairymen were complaining that

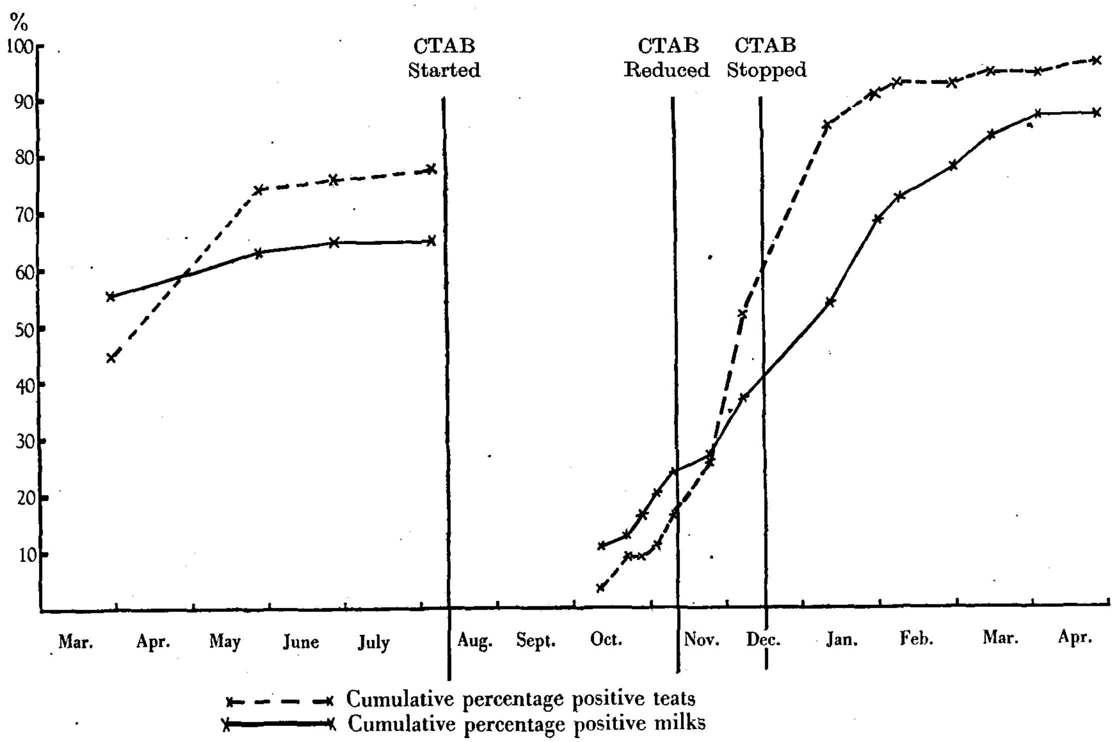

Fig. 1. Graph showing cumulative percentage positive infection rates for teats and milks in 54 cows.

results in two periods, the first before the application of CTAB and the second commencing 2 months later.

It will be noted that there is an interval of 2 months between the commencement of the application of CTAB cream to the milkers' hands and the cows' teats and the first recorded bacteriological examination of the herd. During the interval, bacteriological tests were performed weekly, but a defect in the medium was discovered, and the early results are omitted as their significance is doubtful. However, from 11 October 1943, the results were considered reliable, and they represent the state of the cows were difficult to milk owing to the soreness of their teats. Therefore the amount of CTAB cream applied to the teats and hands was reduced, but the frequency of application was unchanged.

The incidence of teat lesions continued to rise and the teat infection rate also began to increase, until by 7 December the percentage infection was 44·2 % and the cumulative percentage positive teat rate was 51·8 %.

The milk infection rate had also altered unfavourably, although the cumulative percentage positive rate was not climbing so rapidly as the teat rate.

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By 15 December it was obvious that the condition of the teats was so badly affected that the application of 1 % CTAB cream had to be abandoned. The teat lesions persisted throughout the winter, and it was only with the return of better weather that their condition improved.

Both the teat and milk rates continued to climb, until by 27 April the cumulative percentage positive rate for teats was 96·2 and 87 % for milk.

DISCUSSION

Although by *in vitro* experiments it was possible to select CTAB as a disinfectant with a high bactericidal action on *Str. agalactiae*, its application for the prevention of the dissemination of infection in a dairy herd did not give encouraging results.

The results of the experiment are best analysed by comparing the cumulative infection rates for teat surface infection and milk infection before the application of CTAB and afterwards. These results are represented graphically in Fig. 1.

It will be seen that before treatment, infection was spreading, since the cumulative rate during 4 months rose from 44 to 77 % in the case of teat infection and from 55 to 64 % in the case of milk infection. At the first test made after treatment began, the new cumulative figure started at the low level of 3 % for the teat rate and 11 % for the milk rate which steadily rose during the next 4 weeks to 16 and 24 % respectively. At this time it was observed that abnormalities of the teat were being produced, and in spite of reducing the amount of dressing applied, the infection rates rose to 51 and 37 %, which were succeeded by very high rates after discontinuing treatment.

The experiment showed, therefore, that hand disinfection produced an immediate reduction in the infection of the teat surface after application and a lowered rate of spread for a short period, but this satisfactory result was completely changed by the development of sores and fissures on the teats of the cows which was accompanied by a simultaneous increase in both the teat and milk infection rates. Since these high rates were subsequently maintained for some months, it is clear that wide dissemination of infection had occurred in the herd. The repeated application of CTAB, even though it was incorporated in an oily base, must be held responsible, in part, for the condition of the teats, since Williams has also noted that a sensitivity of the hands may develop as a result of applying 1 % CTAB in water repeatedly. Of the milkers employed in this herd during the experiment, only one developed 'chaps' of the hands which might have been attributed to CTAB.

This undesirable result of applying a disinfectant to the cows' teats must also be aggravated by exposure in winter to cold, drying winds, because it is a fairly common experience that 'chapped' teats are more prevalent in winter time.

The results of these experiments show, therefore, that the application of disinfectant in a strength which is sufficient to destroy *Str. agalactiae* may also be injurious to the skin of the teat, and that lesions of the teat established in this way provide suitable foci of infection in which organisms are resistant to the application of a disinfectant.

CONCLUSIONS

1. The application of 1 % CTAB in a lanette wax-oil base, twice daily, to the teats of cows and the milkers' hands for over 2 months at the time of milking in a dairy herd appeared to reduce the spread of infection by *Str. agalactiae*.

2. After application for 3 months, lesions of the teats developed, accompanied by a rapid increase in the infection of the teats and milk with *Str. agalactiae*. 1 % CTAB cream failed to control this increase in infection.

3. It is suggested that, at least in part, the increase in the number of cows with teat lesions was due to the use of CTAB.

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