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Observations on Bacterial Activity in Natural Rubber Latex — Plate Counts of Latex Bacteria on a Supplemented Medium

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Observations on field latices preserved with ammonia and zinc dialkyl dithiocarbamate sometimes showed rapid development of volatile fatty acids (VFA) when the latex was virtually sterile. This was contrary to general experience and following a similar observation by other workers it was found that by using a culture medium containing latex serum and ammonia, much higher bacterial counts were obtained on these latices than with the yeast extract agar normally employed.

Using both media for culturing bacteria has demonstrated the occurrence of ammonia-loving bacteria in field latex and in ammoniated concentrates.

It is suggested that both types of medium are required for plate cultures to obtain a complete picture of bacterial populations in latex.

Ammonia-loving bacteria are considered important because they proliferate in the presence of ammonia and can be strong producers of VFA; they may account for the coagulation of apparently 'good' latex concentrates after a few weeks storage.

Experience in the natural rubber industry indicates that when field latex or latex concentrate is adequately preserved, deterioration in properties on storage occurs only very slowly. Adequate preservation is normally attained by using ammonia or caustic potash, either alone or with the addition of further bactericides, enzyme poisons, metal ion sequestering agents and so on. Several tests for assessing the condition of a given latex are known and one commonly used for indicating the degree of spoilage is to measure the level of volatile fatty acids (VFA) present; these are known to be produced from carbohydrates and other substances naturally occurring in latex by the metabolic activity of proliferating bacteria. A well preserved latex shows little or no change in VFA during storage. General experience shows that VFA are found only when bacterial activity is present or has already occurred. In some cases during storage a very slow and small rise occurs; this is regarded as negligible, and it is a moot point whether it results from microbial, enzymic or chemical activity. However, all major increases in VFA are normally found to be associated with bacterial proliferation. Experimental studies confirming these observations were made by Taysum[1] who showed that latex concentrate free from viable bacteria need have a VFA number no greater than 0.007 after one month's storage. At the other extreme John[2] showed that acids produced by the action of adventitious bacteria which metabolise indigenous non-rubber substances in latex can bring about spoilage by destabilisation and coagulation.

It must be pointed out, however, that not all bacteria occurring in latex produce VFA; some are strong producers of acid (SAP), others not[3]. The use of indicators registering changes in the pH value of plating media clearly show that only certain colonies cul-

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tured from latex bacteria are acid-producing[4]. The presence of a large number of bacteria found in a given latex, for example by making a plate count, is thus not a completely reliable indication of high VFA production; the reverse statement that when large amounts of VFA are formed high levels of bacterial activity are necessarily associated would, however, appear to be sound.

Recent observations in these laboratories have shown rapid development of VFA in certain latex concentrates found to be virtually sterile by routine bacterial plate counts; in view of the above discussion the findings were anomalous and form the subject of this paper.

OBSERVATIONS

Typical changes in the levels of VFA and associated bacterial counts occurring in lightly ammoniated field latex on storage are shown in *Table 1.*

It will be noted that even with high levels of bacterial activity (ca.10^6) it requires two days before high levels of VFA (*i.e.* $>$ 0.20) are produced; this could possibly be due to the fact that on storage the proportion of SAP bacteria increases markedly relative to other types[5].

When field latex is preserved with ammonia together with a secondary preservative zinc dialkyl dithiocarbamate), formation of VFA is delayed. Typical data are as given in *Table 2.*

These observations indicate that large amounts of VFA are being formed in the latex after the bacteria have been killed off. For example the latex from bulk tank No. 3 shows an increase of VFA from 0.021 to 0.346 during a period when the bacterial counts, (19, nil, 11) showed the latex to be virtually sterile. At face value such observations did not appear to be acceptable and obviously required investigating.

A survey of the literature revealed no evidence that sizeable quantities of VFA can be produced in latex by means other than microbial activity. An interesting paper by Rhines and McGavack[6], however, describes observations made on Malaysian latex received in America showing similarity to the observations above. It was found that certain shipments of ammoniated latex concentrates had poor stability and bad odour, indicative of microbial spoilage, yet bacteriological checks carried out in various places repeatedly classified the latex as sterile. The discrepancy was eventually traced to the use of an unsuitable medium for culturing the bacteria, the authors demonstrating that ammonia-loving bacteria were responsible for the latex spoilage, and that these required a medium containing ammonia if they were to be cultured successfully. Plating on a normal medium thus showed a spurious 'nil' count

TABLE 1. TYPICAL CHANGES IN VFA NUMBER AND BACTERIAL COUNTS IN FIELD LATEX DURING STORAGE

Item	Storage time	Bacterial count	VFA no.
Field latex ammonia 255 meq. (0.43% on latex)	Day 0	1.0×10^7	0.027
	Day 1	8.4×10^7	0.116
	Day 2	9.6×10^7	0.290
	Day 4	8.7×10^5	0.870
	Day 7	3.0×16^6	1.030
Field latex ammonia 270 meq. (0.46% on latex)	Day 0	8.7×10^6	0.025
	Day 1	7.9×10^5	0.037
	Day 2	1.7×10^7	0.210
	Day 4	6.5×10^7	0.850
	Day 7	3.4×10^7	1.060

TABLE 2. CHANGES IN VFA NUMBER AND BACTERIAL COUNTS IN FIELD LATEX PRESERVED WITH AMMONIA AND A SECONDARY BACTERICIDE

Bulk tank	Preservative used	Storage time	Bacterial count[a]	VFA no.
No. 1	Ammonia 250 meq. zinc dialkyl dithiocarbamate (ZDD) 0.1 phs	Day 0	6.3×10^5	0.022
		Day 1	3.8×10^4	0.027
		Day 2	330	0.047
		Day 4	27	0.048
		Day 7	6	0.245
No. 2	Ammonia 250 meq. ZDD 0.1 phs	Day 0	3.6×10^5	0.017
		Day 1	2 400	0.018
		Day 2	110	0.017
		Day 4	40	0.020
		Day 7	nil	0.092
No. 3	Ammonia 250 meq. ZDD 0.1 phs	Day 0	1.9×10^6	0.018
		Day 1	6 300	0.020
		Day 2	19	0.021
		Day 4	nil	0.085
		Day 7	11	0.346
No. 4	Ammonia 275 meq. ZDD 0.1 phs	Day 0	7.6×10^5	0.033
		Day 1	2.4×10^4	0.038
		Day 2	58	0.040
		Day 4	nil	0.182
		Day 7	nil	0.260
No. 5	Ammonia 275 meq. ZDD 0.1 phs	Day 0	1.6×10^6	0.025
		Day 1	3.2×10^4	0.020
		Day 2	2.6×10^4	0.024
		Day 4	13	0.087
		Day 7	17	0.470

[a]Viable cells per millilitre latex

whereas plating in an ammoniated medium showed the latex to be 'teaming with bacteria'.

EXPERIMENTAL

The medium we had been using in our laboratory for making plate counts of viable bacteria followed that put forward by John[7] which was based on molasses and yeast extract (MYE) with additions to suppress mould growth*. This was shown by John to be simpler to prepare but equivalent to the modified Kligler's iron agar developed by Taysum[8] in respect of bacteria occurring in fresh field and ammoniated latices. Taysum had shown his medium to be simpler to prepare and equivalent to the sterilised latex serum originally used by Overton[9]. Whilst there is no reason to doubt the suitability of the media developed by Taysum and by John for normal latex bacteria, the work of Rhines and McGavack suggests that the addition of low levels of ammonia to a culture medium may be essential if ammonia-loving bacteria, *i.e.* those requiring ammoniated latex serum for active proliferation, are to be grown successfully. Trials were therefore made using culture media with and without the addition of the supplements described by Rhines and McGavack, *viz* ammonia, glucose and sterilised latex serum.

The media used were modified yeast extract agar[7] and nutrient agar, details being

*For routine use, the addition of mould suppressants was found to be unnecessary.

given in the *Appendix* which also records the levels of ammonia and other materials used as supplements. Plating procedure followed that recommended by the RRIM[8,10] using 1 ml quantities of ten-fold serial dilutions. Incubation was carried out for five days at 30°C.

Measurement of VFA number was made according to the method of British Standard Specification No. 1672.

RESULTS

The first trial, recorded in *Table 3*, was carried out on field latex from a factory bulk tank, samples being plated out on four media, molasses yeast agar and nutrient agar each with and without the Rhines and McGavack supplements. The observations clearly indicated a high level of bacterial activity in the latex as judged by the rate of VFA production, but both the unsupplemented media showed low bacterial counts, nil to 400 cells per millilitre, during the period when most of the VFA was produced. With ammonia-supplemented nutrient agar, however, high counts up to 1×10^6 cells per millilitre were found for this active period.

A further trial, using samples of field latex taken directly from estate collecting stations and ammoniated to 300 meq (0.51% on latex) was carried out comparing supplemented (sterilised latex serum, glucose and ammonia) nutrient agar (SNA) with two other unsupplemented media, molasses yeast extract agar[7] and modified Kligler iron agar[8].

Table 4 shows that latices from *Estates A* and *C* were well preserved with very low VFA formation. The unsupplemented media showed some bacterial activity which dropped to zero after four days; the supplemented medium showed no ammonia-loving bacteria. In the case of *Estate B* latex, however, VFA developed rapidly from two to four days; both types of media showed bacterial activity, that in the supplemented medium being one to two orders higher as VFA was produced.

Similar tests were made on samples of latex taken at the factory from lorry tankers delivering from *Estates D* and *E* as shown in *Table 5*. Counts an order higher were found on the supplemented medium for latex from *Estate E* as development of VFA occurred.

Further tests carried with preserved field latex from different bulk tanks are shown in *Table 6*. In each case VFA was developing rapidly and counts on the supplemented medium were one to two orders higher than on the unsupplemented ones.

At this stage although it appeared that the presence of latex serum and ammonia were playing an important role in culturing ammonia-loving bacteria, it was possible that the higher alkalinity of the medium was the important feature, rather than the actual presence of ammonia or ammonium salts. The data given in *Table 7* compare bacterial

TABLE 3. BACTERIAL COUNTS OF FACTORY BULK LATEX USING DIFFERENT CULTURE MEDIA

Medium	Viable bacteria per millilitre latex at different storage times				
	Reception	1 Day	2 Days	4 Days	7 Days
Molasses yeast agar	7.1×10^5	2.4×10^4	3.8×10^2	89	Nil
Molasses yeast agar + latex serum + glucose + NH_3	4.3×10^3	1.4×10^3	3.0×10^2	60	14
Nutrient agar	8.1×10^5	3.6×10^4	4.0×10^2	79	Nil
Nutrient agar + latex serum + glucose + NH_3	6.5×10^3	4.0×10^3	5.4×10^2	1.1×10^5	1.1×10^6
VFA content observed in latex	0.020	–	–	0.067	0.162

TABLE 4. BACTERIAL COUNTS OF LATEX FROM ESTATE COLLECTING STATIONS USING DIFFERENT CULTURE MEDIA

Latex source	Medium	Viable bacteria per millilitre latex at different storage times			
		Reception	1 Day	2 Days	4 Days
Estate A	Molasses yeast extract agar	4.1×10^{6}	2.7×10^{3}	1.8×10^{2}	35
	Modified Kligler iron agar	5.8×10^{6}	2.3×10^{3}	2.9×10^{2}	12
	Supplemented nutrient agar (NH_3 etc.)	Nil	Nil	Nil	Nil
	VFA content of latex	0.013	0.010	0.015	0.013
Estate B	Molasses yeast extract agar	6.3×10^{7}	4.1×10^{4}	3.0×10^{3}	5.2×10^{5}
	Modified Kligler iron agar	4.7×10^{7}	3.8×10^{4}	4.5×10^{3}	1.2×10^{5}
	Supplemented nutrient agar (NH_3 etc.)	5.5×10^{4}	5.0×10^{3}	5.1×10^{4}	2.5×10^{7}
	VFA content of latex	0.019	0.020	0.033	0.268
Estate C	Molasses yeast extract agar	1.5×10^{5}	4.8×10^{3}	4.8×10^{2}	54
	Modified Kligler iron agar	3.6×10^{5}	4.1×10^{3}	4.9×10^{2}	20
	Supplemented nutrient agar (NH_3 etc.)	Nil	Nil	Nil	Nil
	VFA content of latex	0.007	0.012	0.009	0.017

TABLE 5. BACTERIAL COUNTS OF LATEX FROM INCOMING LORRY TANKERS USING DIFFERENT CULTURE MEDIA

Latex source	Medium	Viable bacteria per millilitre latex at different storage times			
		Reception	1 Day	2 Days	4 Days
Estate D	Molasses yeast extract agar	5.9×10^{4}	1.7×10^{4}	8.5×10^{3}	2.0×10^{3}
	Modified Kligler iron agar	1.1×10^{5}	1.3×10^{4}	7.8×10^{3}	3.1×10^{3}
	Supplemented nutrient agar (NH_3 etc.)	1×10^{3}	1.4×10^{3}	5.2×10^{3}	1.9×10^{4}
	VFA content of latex	0.023	0.018	0.025	0.031
Estate E	Molasses yeast extract agar	7.6×10^{5}	3.5×10^{5}	3.2×10^{4}	3.4×10^{6}
	Modified Kligler iron agar	1.4×10^{6}	6.0×10^{5}	3.7×10^{6}	3.2×10^{6}
	Supplemented nutrient agar (NH_3 etc.)	1.4×10^{4}	7.6×10^{4}	2.3×10^{7}	6.2×10^{7}
	VFA content of latex	0.031	0.031	0.537	0.770

growth on media in which pH was modified from the nominal 7.8 to 9.8, both by potassium hydroxide and by ammonia, in the presence and in the absence of dextrose and latex serum (the latter prepared by freezing and thawing field latex containing no ammonia). The results indicated that the presence of ammonia was essential for active proliferation and was augmented by the presence of latex serum.

TABLE 6. BACTERIAL COUNTS OF DIFFERENT FACTORY BULK LATICES USING DIFFERENT MEDIA

Latex source	Medium	Viable bacteria per millilitre latex at different storage times: Reception	1 Day	2 Days	4 Days
Bulk tank A	Molasses yeast extract agar	3.0×10^6	7.4×10^5	6.0×10^5	4.0×10^6
	Modified Kligler iron agar	2.9×10^6	5.4×10^4	$>1.0\times10^6$	9.3×10^5
	Supplemented nutrient agar (NH_3 etc.)	9.1×10^5	4.5×10^5	$>1.0\times10^6$	9.3×10^7
	VFA content of latex	0.019	0.040	0.215	0.660
Bulk tank B	Molasses yeast extract agar	2.2×10^6	1.8×10^5	1×10^6	1.4×10^6
	Modified Kligler iron agar	7.2×10^5	2.9×10^5	8.7×10^5	9.6×10^5
	Supplemented nutrient agar (NH_3 etc.)	5.2×10^3	1.3×10^4	1.8×10^6	8.1×10^7
	VFA content of latex	0.017	0.019	0.150	0.950
Bulk tank C	Molasses yeast extract agar	1×10^6	1.5×10^5	7.9×10^4	8.3×10^5
	Modified Kligler iron agar	1×10^6	1.5×10^5	1.7×10^5	7.9×10^4
	Supplemented nutrient agar (NH_3 etc.)	5.5×10^3	4.2×10^4	1.3×10^7	4.1×10^7
	VFA content of latex	0.027	0.027	0.127	0.680

TABLE 7. BACTERIAL COUNTS ON FIELD LATEX USING DIFFERENT CULTURE MEDIA

Preservative system	Medium	Viable bacteria per millilitre latex at different storage times: 4 Days	6 Days	8 Days	11 Days	14 Days
Field latex from factory bulk tanks adjusted to:	Nutrient agar					
	pH 7.8 KOH	110	120	60	30	70
	pH 9.8 KOH	70	100	20	90	40
	pH 9.8 NH_3 [a]	110	3.2×10^4	7.2×10^4	6.2×10^3	1.2×10^3
0.43% NH_3)	Nutrient agar + Dextrose + *Serum*					
0.034% ZDD)	pH 7.8 KOH	20	<10	40	70	50
	pH 9.8 KOH	50	70	30	240	30
	pH 9.8 NH_3 [a]	1.0×10^6	4.0×10^5	3.5×10^6	3.0×10^5	1.3×10^6
	Molasses yeast extract					
	pH 7.8	90	110	70	90	20
	VFA content of latex	0.078	0.108	0.125	0.140	0.152

[a]Incubated in an ammoniated atmosphere

It thus became apparent that a medium supplemented with ammonia and latex serum was necessary if certain of the bacteria-producing VFA were to be cultured for plate counting. Such a medium (SNA) was then adopted in routine bacterial counts on field

latex and concentrates in addition to the one (MYE) previously employed and has proved to be valuable in distinguishing between normal and ammonia-loving types of bacteria. The following examples have been selected to demonstrate the value of the supplemented medium (SNA) in indicating the presence of ammonia-loving bacteria which would have remained undetected by the unsupplemented medium (MYE).

Bacterial counts were required on samples of field latex containing ammonia and a secondary preservative for centrifuging. *Table 8* records the counts obtained in routine tests with the normal MYE and supplemented SNA media. *Sample 1* had only a few bacteria present which became inactive in less than a week, and were apparently not ammonia-loving since counts were lower in the ammoniated medium, SNA. VFA did not develop. In *Sample 2,* taken on a different day, ammonia-loving bacteria were obviously present and proliferated at the expense of 'ordinary' bacteria; thus the count on normal medium MYE fell rapidly to zero after four days, whereas that on the supplemented medium SNA increased from two days onwards as the ammonia-loving organisms developed to counts well over 1×10^5. Considerable amounts of VFA were produced as these organisms proliferated. *Sample 3* showed a similar situation.

In order to assess the susceptibility of high ammonia (HA) latex concentrates to contamination during transport, tests were made by inoculating the final product, which had nil bacterial count, with an infected HA concentrate, at a 10% level. In one experiment the effect of adding sodium pentachlorophenate (SPP) to the concentrate was examined with the hope of increasing its resistance to contamination.

The observations in *Table 9* show that the bactericidal power of the straight ammonia concentrate was insufficient to cope with the inoculum received; VFA developed after one or two months. The bacterial count on

TABLE 8. BACTERIAL COUNTS ON FIELD LATEX WITH SECONDARY PRESERVATIVE

Preservative system[a]	Culture medium	Viable bacteria per millilitre latex during storage					
		Reception	1 Day	2 Days	4 Days	1 Week	2 Weeks
Sample 1	MYE	1.4×10^6	2.0×10^5	170	17	Nil	–
	SNA	2.7×10^4	8.5×10^3	Nil	Nil	26	–
	(VFA No.)	(0.016)	(0.023)	(0.023)	(0.023)	(0.023)	–
Sample 2	MYE	1.5×10^6	5.8×10^4	1600	Nil	Nil	–
	SNA	2.0×10^5	5.7×10^4	4900	1.8×10^5	4.4×10^5	–
	(VFA No.)	(0.020)	(0.027)	(0.030)	(0.104)	(0.162)	–
Sample 3	MYE	2.0×10^6	4.8×10^4	2100	120	Nil	13
	SNA	2.5×10^5	1.6×10^5	5000	5.2×10^4	3.0×10^4	2.4×10^4
	(VFA No.)	(0.022)	(0.041)	(0.044)	(0.075)	(0.064)	(0.217)

[a] Field latex from factory bulk tank adjusted to:
0.43% NH_3
0.034% ZDD

Figures within brackets show VFA numbers.

TABLE 9. BACTERIAL COUNTS ON HIGH AMMONIA LATEX CONCENTRATE CONTAINING SODIUM PENTACHLOROPHENATE (SPP) AFTER INOCULATION

Preservative system	Culture medium	Viable bacteria per millilitre latex					
		Before inoculation	Time after inoculation				
			3 Days	2 Weeks	1 Month	2 Months	3 Months
0.8% NH_3	MYE	Nil	18	Nil	Nil	Nil	Nil
	SNA	38	1.0×10^5	1.1×10^5	3800	1.8×10^4	7.8×10^4
	(VFA No.)	(0.009)	(0.017)	(0.040)	(0.110)	(0.123)	(0.125)
0.8% NH_3)	MYE	Nil	50	Nil	Nil	Nil	Nil
)	SNA	38	8.1×10^4	9.8×10^4	Nil	4500	1.0×10^6
0.016% SPP)	(VFA No.)	(0.009)	(0.017)	(0.031)	(0.095)	(0.112)	(0.120)
0.8% NH_3)	MYE	30	23	Nil	Nil	Nil	Nil
)	SNA	23	9.3×10^4	8.0×10^4	240	170	5.8×10^4
0.031% SPP)	(VFA No.)	(0.009)	(0.014)	(0.029)	(0.050)	(0.052)	(0.055)
0.8% NH_3	MYE	25	Nil	Nil	Nil	Nil	Nil
	SNA	23	9.8×10^4	2.5×10^5	Nil	140	Nil
0.062% SPP	(VFA No.)	(0.009)	(0.016)	(0.026)	(0.029)	(0.035)	(0.040)
0.8% NH_3	MYE	35	Nil	Nil	Nil	Nil	Nil
	SNA	28	1.1×10^5	7800	Nil	Nil	Nil
0.093% SPP	(VFA No.)	(0.009)	(0.016)	(0.027)	(0.024)	(0.029)	(0.031)

Figures within brackets show VFA numbers.

normal MYE, however, was nil and could thus be very misleading; that on the supplemented SNA showed many active bacteria which increased in numbers from one to three months.

With the addition of SPP at low levels, VFA formation was reduced; the count on the normal medium was still zero, whereas that on SNA was considerable, although lower than without SPP. With higher levels of SPP, VFA production was almost completely suppressed and the bacterial counts on the ammonia-supplemented medium fell to zero, indicating satisfactory preservation.

The lower counts at one and two months shown by the SNA medium for the 0.016% and 0.03% levels of SPP possibly suggest that the ammonia-loving bacteria in the inoculum were adjusting themselves to the somewhat unfavourable new environment of a concentrate containing sodium pentachlorophenate. At higher levels of SPP (0.062% and 0.093%) the ammonia-loving bacteria in the inoculum failed to survive after one month.

In further trials examining the effect of secondary preservatives on bacteria in HA concentrate, a range of substances was examined as set out in *Table 10*. Here again, sterile concentrate was inoculated with 10% of infected latex. Observations on the straight HA concentrate were similar to those in the previous example: VFA was developed, but even so the ordinary medium MYE showed complete absence of active bacteria; the supplemented SNA, however, gave a high level of ammonia-loving organisms, which, within three months, resulted in coagulation of the concentrate. With the addition of low levels of a secondary preservative it was found that SPP had a slight effect

TABLE 10. BACTERIAL COUNTS ON HIGH AMMONIA LATEX CONCENTRATE CONTAINING SECONDARY PRESERVATIVES AFTER INOCULATION

Preservative system	Culture medium	Viable bacteria per millilitre latex					
		Before inoculation	Time after inoculation				
			3 Days	2 Weeks	1 Month	2 Months	3 Months
0.8% NH_3	MYE	Nil	Nil	Nil	Nil	–	
	SNA	Nil	1.4×10^4	1.9×10^5	1.3×10^5	1.3×10^6	COAGD
	(VFA No.)	(0.011)	(0.057)	(0.176)	(0.176)	(0.191)	
0.8% NH_3	MYE	Nil	Nil	Nil	Nil	–	–
	SNA	Nil	7800	4.1×10^4	2.2×10^5	1.5×10^6	4.9×10^4
0.016% SPP	(VFA No.)	(0.008)	(0.060)	(0.184)	(0.178)	(0.182)	(0.224)
0.8% NH_3	MYE	Nil	Nil	Nil	Nil	–	
	SNA	Nil	1.2×10^4	3.3×10^5	5.0×10^5	5.6×10^5	COAGD
0.016% OPP	(VFA No.)	(0.012)	(0.057)	(0.191)	(0.184)	(0.232)	
0.8% NH_3	MYE	Nil	Nil	Nil	Nil	–	–
	SNA	Nil	2.8×10^4	3.1×10^5	1×10^6	5.7×10^5	5.9×10^5
0.016% PCMC)	(VFA No.)	(0.017)	(0.074)	(0.193)	(0.194)	(0.218)	(0.279)
0.8% NH_3	MYE	Nil	Nil	Nil	Nil	–	–
0.016% TMTD)	SNA	Nil	2200	1300	Nil	Nil	Nil
0.016% ZnO	(VFA No.)	(0.016)	(0.029)	(0.026)	(0.038)	(0.048)	(0.058)

Figures within brackets show VFA numbers.
SPP = Sodium Pentachlorophenate
OPP = Sodium orthophenylphenate
PCMC = Parachloro metacresol
TMTD = Tetramethyl thiuramdisulphide

in reducing both VFA production and bacterial counts on SNA medium; sodium orthophenylphenate and para-chloro meta-cresol had little effect. Tetramethylthiuram disulphide with zinc oxide reduced VFA formation very considerably and gave a nil count on the SNA medium within one month. In all cases the unsupplemented medium showed nil counts, bacterial activity being revealed only by the supplemented medium.

In making inoculation trials for assessing the effectiveness of preservation in a given latex, it would be desirable to make use of a standard inoculum of bacteria. However, the preparation and maintenance of bacteria to supply such an inoculum is a tedious and time-consuming operation, and a convenient compromise is to maintain a given mixed bacterial population from a 'bad' latex by passaging it to a fresh supply of sterile latex every few days, using this as an inoculum as required. It is, however, important to maintain the ammonia at a constant chosen level, since the organisms adjust themselves to this in the course of developing 'resistance' or 'acclimatisation' to their surroundings.

In one trial bacteria taken from lightly ammoniated field latex, which had been allowed to 'go bad', were developed and maintained in two HA concentrates, one containing 0.2% NH_3, the other 0.7% NH_3; coagulation was avoided by daily passaging to fresh concentrates. A 10% inoculum

from each of these cultures was eventually made into sterile HA concentrate and observations made as given in *Table 11.* As was to be expected, bacterial counts were much higher on the ammoniated SNA medium than on the normal MYE. It is of interest to note that the level of VFA produced with bacteria acclimitised to 0.2% NH_3 was two or three times greater than that developed from those acclimatised to 0.7% NH_3, in spite of the fact that the latter were present in much greater numbers as shown by the SNA medium. This suggests that bacteria developing in low levels of ammonia (0.3% on the *aqueous phase)* may be stronger acid-producers than those developing at higher levels (1.1% on the *aqueous phase).*

DISCUSSIONS AND CONCLUSIONS

Certain bacteria which are commonly present in both field latex and latex concentrate do not produce colonies when plated out on the routine media normally used for this purpose. The presence of ammonia in the substrate appears to be necessary before such organisms can actively proliferate in the normal manner. By using culture media suitably supplemented with ammonia, such as that described by Rhines and McGavack[6], development on plating appears to occur readily. This observation should not be taken to imply, however, that all types of ammonia-resistant or ammonia-loving bacteria require ammonia for active proliferation; very little is known regarding such organisms.

The use of an ammonia-supplemented medium for making plate counts on latex does not appear to be sufficient by itself to give a complete picture of the bacterial situation. Ammonia is known to inhibit the growth of many species comprising the normal bacterial populations of latex. Plate counts on an ammonia-supplemented medium are thus unlikely to give a true representation of 'normal' bacteria present (see *Table 4*). It appears advisable to examine latex by plating out on two media, one with and one without ammonia; by such means a more accurate assessment of the bacterial population may be obtained.

The ammonia-loving bacteria observed in the present study have been found both in latices preserved with ammonia and in those with ammonia and a secondary preservative. The bacteria have been observed to proliferate actively during storage of latex whilst the normal population dies off *(e.g. Table 3).* They can be strong producers of VFA *(e.g.*

TABLE 11. BACTERIAL COUNTS ON HIGH AMMONIA LATEX INOCULATED WITH AMMONIA-RESISTANT BACTERIA

Type of inoculum	Culture medium for plating	Viable bacteria per millilitre latex							
		Before inoculation	Time after inoculation						
			Same day	1 Day	2 Days	4 Days	1 Week	2 Weeks	4 Weeks
From field latex containing 0.2% NH_3	MYE	Nil	7.8×10^6	2.7×10^6	8.0×10^4	4 900	870	7 400	8 800
	SNA	Nil	5.5×10^6	1.3×10^6	3.0×10^6	3.6×10^6	1×10^7	3.3×10^6	1.1×10^4
	(VFA no.)	(0.027)	(0.091)	(0.104)	(0.122)	(0.124)	(0.196)	(0.324)	(0.448)
From field latex containing 0.7% NH_3	MYE	Nil	1.3×10^4	8 100	130	16	8	Nil	Nil
	SNA	Nil	1.3×10^5	1.1×10^5	3.1×10^5	4.6×10^6	5.2×10^7	3.8×10^7	3.7×10^7
	(VFA no.)	(0.027)	(0.032)	(0.037)	(0.045)	(0.061)	(0.060)	(0.078)	(0.154)

Figures within brackets show VFA numbers.

Tables 3, 6, 8 and *11*). It is thought that their presence may account for a phenomenon sometimes observed by latex concentrate producers: a bulk of latex showing low bacterial count (by normal plating techniques) and low rate of VFA formation, which are regarded as reasonably satisfactory, may after a few weeks' storage suddenly develop high levels of VFA, leading on occasions to spoilage of the latex. Data in *Table 10* exemplify the phenomenon of delayed coagulation.

An important corollary of this study is that latices showing a nil bacterial count on normal plating media may not necessarily be sterile; to regard them as such may be misleading particularly when assessing future behaviour of the latices. It is possible that a low rate of VFA formation may also be deceptive. In the present state of the art, latex sterility in respect of both normal and ammonia-loving bacteria together with a low rate of VFA formation would appear to be the best safeguard. Even then the possibility of contamination during bulk shipment still exists. The ability of the preservative system to cope with inocula of both normal and ammonia-loving bacteria during transit is thus of major importance and should be checked prior to despatch, if satisfactory storage and shipment is to be ensured.

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APPENDIX

The three basic media used in this work were as follows:

Molasses Yeast Agar (MYE)	Percent
Oxoid agar No. 3	1.25
Molasses	0.5
Yeast extract	0.5
Bromocresol purple	0.005
Nutrient Agar	
Oxoid agar No. 3	1.5
'Lab-lemco' beef extract	0.1
Yeast extract	0.2
Peptone	0.5
Dextrose	0.5
Sodium chloride	0.5
Modified Kligler Iron Agar	
Oxoid agar No. 3	1.2
'Lab-lemco' beef extract	0.3
Yeast extract	0.3
Peptone	2.0
Lactose	1.0
Dextrose	0.2
Meso-inositol	0.3
Sodium chloride	0.5
Ferric citrate	0.03
Sodium thiosulphate, $5H_2O$	0.03
Phenol red	0.005

They were prepared with distilled water, adjusted to pH 7.8 and sterilised in the usual way.

For supplementing culture media according to the Rhines and McGavack method, sterile latex was prepared by ammoniating field latex (33% to 34% d.r.c.) to 1.25%, *i.e.* 1.9% on the aqueous phase, adding sufficient ammoniate alginate to give 0.15% on the water phase and allowing the mixture to cream overnight. The clear serum was separated off, mixed with a 20% dextrose solution in the proportion 10:1 and heated at 60°C for ½h to 1h by which time it was usually found to be sterile; it was tested by plating on nutrient agar. If not found sterile, further heating was applied. To prepare the supplemented media, the sterile serum/detrose mixture eleven parts was stirred into forty parts of the melted prepared medium (molasses yeast extract agar or nutrient agar). The final medium had a pH value of 9.3. In making bacterial counts a control plate of the supplemented agar was incubated together with those receiving inocula for testing. The control plate normally showed a nil bacterial count; in those cases where the control plate was not sterile the test was discarded and a new series of plates prepared.