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Errors in fat testing of composite milk samples

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(*Received* 5 *December* 1967)

Summary. Analyses were made at the Institute chemistry laboratory over 2 dairy seasons of 900 composite milk samples prepared for routine fat testing at 2 dairy factories and held at ambient temperature. The composites, which were preserved with mercuric chloride, frequently had high free fat acidities and high bacterial and mould counts.

At one factory, where the composites were sampled at 85–90 °F, the samples contained incompletely dispersed fat. Werner Schmid fat values for 83 milks pipetted immediately after factory sampling were lower on average by $0{\cdot}084 \pm 0{\cdot}089$ % than values obtained for the same samples after high-speed mixing at 115 °F. At the other factory, where the samples were mixed at 98–103 °F, Werner Schmid tests on 56 milks showed a corresponding difference of only $0{\cdot}033 \pm 0{\cdot}047$ %.

For 273 factory composites, mixed at high speed, results by the Babcock test and by the Gerber test averaged, respectively, $0{\cdot}015 \pm 0{\cdot}019$ % and $0{\cdot}059 \pm 0{\cdot}054$ % higher than those by the Werner Schmid test.

High bacterial and mould counts in factory composites were attributed to contamination from unsterilized bottles and stoppers and to failure of factory staff to mix the contents of each bottle after the daily addition of milk. Low bacterial and mould counts and negligible increases in free fat acidity were found in composites made up from factory milk in the Institute laboratory in sterilized bottles, kept at ambient temperature and swirled daily. Composites made up from fresh farm milks and held at 38–40 °F remained in good condition for sampling after warming to 98–103 °F but showed wide variation in free fat acidity.

Rises in free fat acidity occurred only in composites preserved with mercuric chloride. Other preservatives, however, were less effective in preventing bacterial growth at ambient temperatures.

Increases in free fat acidity up to 10 (ml N alkali/100 g fat) had no effect on results in the Babcock tests but caused a slight rise with the Gerber tests. Acidities over 20 caused a decrease in Babcock and a further rise in Gerber values.

It is suggested that fat destabilization in composite samples could be minimized by avoiding rises in temperature during transport of fresh daily samples from farm to factory, by holding composites at 55–60 °F and by mixing after each daily addition of milk.

Babcock fat tests on composites made up from samples of milk supplied each day over a 10-day period are used in all New Zealand dairy factories to estimate the quantity of butterfat to be credited to each supplier.

There are many references in the literature to slight decreases in the Babcock fat test values of composite samples preserved with mercuric chloride in comparison with the average of the values for the daily milks making up the composite (Taylor & Stull, 1962). That the decreases, which have varied considerably from one investigator to another, were due partly at least to the method of handling and storing the composites was shown by Wilster & Robichaux (1940) who found an average reduction of only 0·027% in 60 7-day composites kept at 35–40 °F and as high as 0·15% in composites in unsterilized bottles kept at room temperature and poorly mixed each day. The authors found that the reduction also applied to the Mojonnier (modified Röse–Gottlieb) test which showed a decrease of 0·021% on 60 7-day composites and of 0·055% on the same number of 15-day composites. Since these samples had been handled, stored and sampled under controlled conditions, the authors considered that the reduction was not due to sampling difficulties. Holland (1938) also reported a progressive decline in both Babcock and Mojonnier tests on composite samples, but explained the reduction as being due to poor dispersion of the fat because of clumping, churning, and oiling-off of the fat globules.

Composite samples tested by Manus & Bendixen (1956) also showed a drop in the Babcock test proportionate to the increase in free fat acidity of the milk. Since with other preservatives there was little change either in free fat acidity or in apparent fat content, it appeared that mercuric chloride stimulated the lipolytic hydrolysis of the fat. An explanation for the drop in fat test that covers both the increase in fat acidity and the error in sampling has been advanced by Herrington (1964). He suggests that mercuric chloride denatures the protein of the fat globule membrane, liberating some of the fat which is thus exposed to attack by lipase, and also causing partial breaking of the emulsion which, in turn, leads to clumping and churning.

The present paper describes an investigation into the condition of composite samples used for routine fat testing at 2 dairy factories where the samples were handled, stored and tested under conditions typical for many dairy factories in New Zealand.

Composites made up in the Institute laboratory and the Institute experimental dairy were also examined.

EXPERIMENTAL

Composite samples

Factory samples

Over the 2 dairy seasons 1964–5 and 1965–6 a large number of composite samples used for routine fat testing in 2 dairy factories were analysed at the Institute chemistry laboratory. The composite samples were made up by the addition each day to the collecting bottle of a dipperful (approximately 15 ml) of fresh milk from the ½-pint sample taken as the contents of each farm tank were being transferred to the tanker, with no further mixing. At the start of the collection period 2 tablets of preservative each containing, according to the manufacturer's label, 0·13 g mercuric chloride, were placed in each collecting bottle. All the composite samples were kept on shelves on

the factory receiving stage (factory A) or in the factory test room (factory B), unprotected from light. At the end of the 10-day period the composites at factory A were heated to 85–90 °F as recommended in New Zealand Department of Agriculture instructions for fat testing (1958), and those at factory B to 98–103 °F, and mixed before testing. After withdrawal of the milk required for the factory Babcock test, further samples, if required, were measured out for testing at the Institute laboratory. The remaining portions of the composite samples were then brought to the laboratory, heated to 115 °F and emulsified with a high-speed mixer (Polytron) so that any churned or free fat was uniformly incorporated into the sample. Each sample was allowed to stand for a short time for air to escape. The bottles were then gently inverted and samples were withdrawn at about 80 °F for estimation of fat content and free fat acidity.

Laboratory samples

On 4 occasions (in April, June and November 1965, and in February 1966) 2 composite samples similar in volume and quantity of preservative to the factory composites were made up in the laboratory from daily samples of fresh milk from factory A. All the composites were kept at laboratory temperatures and were not protected from light. After each daily addition of fresh milk, the collecting bottle was swirled gently to mix the contents. At the end of 10 days each composite was warmed to 115 °F and emulsified with a high-speed mixer before analysis.

Experimental dairy samples

Composite samples were made up from the milk supplied by 2 nearby farms to the Institute experimental dairy over the period January–May 1965. The quantity of preservative and the volume of milk added was the same as that added at the factories, but the composites were all kept at 38–40 °F and not at ambient temperatures. For Babcock testing the composites were warmed to 98–103 °F, mixed and then cooled. These samples were in such good condition that no further heating and mixing was required before sampling in the laboratory for the Gerber test.

Lipase-treated milks

Several trials were carried out on the effect of adding 0·1 % (w/v) of a commercial lipase preparation together with mercuric chloride to fresh milk. The fat contents and free fat acidities were estimated on the fresh samples and after storage at 40, 72 and 86 °F.

Bacteriological analysis

Small portions of the factory samples were taken for bacteriological analysis, after mixing but before the withdrawal of milk for fat testing. Plate counts were determined on lactose yeast phosphate agar after incubation at 86 °F for 48 h. Since moulds developed in this medium these also were counted at the same time as the bacterial colonies.

Chemical analysis

Methods for fat estimation

The Babcock test was carried out according to instructions issued by the New Zealand Department of Agriculture (1958) using 10% test bottles graduated in 0·2% divisions; only single estimations were made.

Fat estimations by the Gerber method (British Standards Institution, 1955) were carried out in duplicate. All butyrometers had been checked by the method of Dolby (1949) and showed variations not greater than 0·02%. The Werner Schmid method, on a slightly reduced scale with 8 ml instead of 10 ml quantities of milk, acid and 96% ethanol to allow the use of Mojonnier tubes, was preferred to the Röse–Gottlieb as a standard method for composite milks since in the presence of free fatty acids the recovery of the acids in the former method was so much greater (Walstra & Mulder, 1963).

Free fat acidity in milk

This was estimated by a procedure described by McDowell (1964). Results were expressed as ml N-alkali/100 g fat.

RESULTS

Fat tests

Factory samples

Results for nearly 500 samples from both factories in the 1964–5 season showed that results obtained in the Institute laboratory by the Gerber procedure, after mechanical mixing, were almost always higher than those from the factory test with the Babcock method. Since the difference could have been due to uneven dispersion of fat in the composites under factory sampling conditions, it was arranged that on each test day during the 1965–6 season a measured quantity of milk for a Werner Schmid test should be withdrawn from a number of composites immediately after the withdrawal of the factory Babcock sample. A comparison of the average results for all these composites after factory pipetting and after high-speed mixing is shown in Table 1. Even at factory B, where the difference was much smaller than at factory

Table 1. *Average Werner Schmid results for factory composites sampled at the factory and at the laboratory*

	Factory A	Factory B
No. of samples	83	56
Fat, % (*a*) sampled at factory	4·634	5·067
Fat, % (*b*) sampled in laboratory	4·718	5·100
Difference. (*b*) − (*a*)	+0·084	+0·033*
Standard deviation of difference	±0·089	±0·047

* Significant at 0·1% level.

A, there was statistically a highly significant difference between the results. A frequency distribution of the difference between the the results (see Table 2) shows that at factory A 85·5% and at factory B 76·8% of the samples gave lower fat contents when pipetted at the factory than after high-speed mixing.

The average results for 273 factory composites analysed in the laboratory after high-speed mixing are shown in Table 3. The Babcock result for each factory was closer to the Werner Schmid than the Gerber result, though the standard deviation of the differences from the Werner Schmid are greater for the Babcock than for the Gerber test. The Babcock results obtained by the factory testers were appreciably lower, due in part at least to sampling conditions, and are not quoted.

Table 2. *Frequency distribution of the differences between results for factory composites sampled at the factory and at the laboratory*

		Fat %, sampled at laboratory, minus fat % sampled at factory		
Factory	No. of milks	Range of differences	No. of samples	% of total
A	83	+0·41 to +0·50	1	1·2
		+0·31 to +0·40	2	2·4
		+0·21 to +0·30	3	3·6
		+0·11 to +0·20	18	21·7
		+0·06 to +0·10	22	26·5
		+0·01 to +0·05	25	30·1
		0·00	9	10·8
		−0·01 to −0·05	3	3·6
B	56	+0·11 to +0·20	3	5·4
		+0·06 to +0·10	12	21·4
		+0·01 to +0·05	28	50·0
		0·00	1	1·8
		−0·01 to −0·05	10	17·9
		−0·06 to −0·10	2	3·6

Table 3. *Average Werner Schmid, Babcock and Gerber results for factory composites sampled at the laboratory*

	Factory A	Factory B
No. of milks	144	129
Werner Schmid, fat %	4·561	4·866
Babcock, fat %	4·571	4·888
Difference from Werner Schmid	+0·010	+0·022
Standard deviation of difference	±0·097	±0·085
Gerber, fat %	4·620	4·925
Difference from Werner Schmid	+0·059	+0·059
Standard deviation of difference	±0·059	±0·049
Free fat acidity: Mean	7·82	4·87
Free fat acidity: Standard deviation	±3·52	±2·62
Free fat acidity: Range	1·8–22·6	1·4–19·1

Institute laboratory and experimental dairy samples

The average Werner Schmid, Babcock and Gerber results for the 8 laboratory composite milks were all slightly lower than the respective averages for all the daily milks (see Table 4).

The average Babcock test result for 18 experimental dairy composites, kept at 38–40 °F, was only 0·015 % lower than the average for all the daily milks (see Table 5).

Free fat acidity

The average fat acidity for all the composites at factory A was higher that than for factory B (see Table 3). Since the fat acidity of fresh milk at these factories was unlikely to have exceeded a value of 2·5 there was, in a large proportion of samples, an increase, sometimes considerable, in the free fat acidity of the composites before testing.

The average fat acidity for the laboratory composite samples was the same as that for all the daily milk samples (see Table 4). The fat acidity for the 18 experimental dairy composites held at 38–40 °F varied quite widely (see Table 5) and had an average value of 4·0.

Table 4. *Average Werner Schmid, Babcock and Gerber results for* 8 *laboratory composites compared with the averages for all the daily milks*

	Daily samples (*a*)	Composite samples (*b*)	Differences, (*b*) − (*a*)
Werner Schmid, fat %	4·957	4·938	−0·019
Babcock, fat %	4·961	4·935	−0·026
Gerber, fat %	4·975	4·973	−0·002
Free fat acidity	2·0	2·0	—

Table 5. *Average Babcock and Gerber results for* 18 *experimental dairy composites compared with the average Babcock values for all the daily milks*

		Daily samples (*a*)	Composite samples (*b*)	Differences, (*b*) − (*a*)
Babcock, fat %		5·576	5·561	−0·015
Gerber, fat %		—	5·600	—
Free fat acidity	Mean	1·5	4·0	—
	Range	—	1·4–13·0	—

Effect of increase in free fat acidity on Babcock and Gerber results

The effect of high free fat acidities in factory composites on Werner Schmid, Babcock and Gerber results is shown in Table 6. The average Werner Schmid and Babcock results agreed almost as closely as they did in milks of low fat acidity. The average Gerber result, however, was 0·087% higher than the Werner Schmid at high fat acidities compared with 0·048% at low acidities.

Four of the 18 composite samples from the experimental dairy showed fat acidity values greater than 5·0 (range of values 6·1–13·0). The average Babcock result for these 4 milks was, however, only 0·023% below that for the average of all the daily milks. Since the reduction for 12 of the composites with low free fat acidities (range 1·4–3·1) was 0·017%, the difference at high and low acidities was insignificant.

Composite samples forwarded to the Institute from factories in other districts and examined for fat acidity several days after factory testing showed high values, some as high as 30–40. Part of these increases could have occurred subsequent to sampling at the factory. The amounts of samples were, unfortunately, insufficient for fat testing.

The effect of fat hydrolysis produced by addition of 0·1 % of a commercial lipase to milk preserved with mercuric chloride was studied with 3 milks kept at different temperatures as shown in Table 7. Fat acidities of over 10 caused increases in the Gerber test, but only where the free fat acidity exceeded 20 was there a reduction in the Babcock test.

Table 6. *Average Werner Schmid, Babcock and Gerber results for factory composites of low and high free fat acidity*

	Low free fat acidity	High free fat acidity
No. of milks	32	37
Free fat acidity { Mean	2·2	13·0
Free fat acidity { Range	1·4–2·9	10·1–22·6
Werner Schmid, fat %	4·839	4·657
Babcock, fat %	4·844	4·668
Difference from Werner Schmid	+0·005	+0·011
Gerber, fat %	4·887	4·744
Difference from Werner Schmid	+0·048	+0·087

Table 7. *Effect of addition of lipase on the free fat acidities and Babcock and Gerber results for 3 milks preserved with mercuric chloride*

			Babcock		Gerber	
Sample no.		Free fat acidity	Fat in milk, %	Difference from fresh milk	Fat in milk, %	Difference from fresh milk
1	Fresh	3·4	4·10	—	—	—
	Stored 10 days at 40 °F	10·1	4·10	0·00	—	—
	Stored 10 days at 72 °F	35·1	4·05	−0·05	—	—
	Stored 10 days at 86 °F	44·3	3·95	−0·15	—	—
2	Fresh	2·6	4·60	—	4·60	—
	Stored 10 days at 40 °F	11·5	4·60	0·00	4·68	+0·08
	Stored 10 days at 72 °F	32·3	4·50	−0·10	4·71	+0·11
	Stored 10 days at 86 °F	45·3	4·40	−0·20	4·83	+0·23
3	Fresh	2·2	5·20	—	5·23	—
	Stored 12 days at laboratory temperature	25·2	5·10	−0·10	5·36	+0·13

Bacterial and mould counts

Bacterial counts for a large number of samples from factories A and B varied from 0 to 5 million/ml. Mould counts ranged from 0 to 490000/ml. An independent investigation (H. R. Whitehead, personal communication) showed that the actual amount of mercuric chloride present was 6–8 times that required to destroy all bacteria. The high counts can only be explained by the failure of the factory staff to mix the fresh milk added each day with the milk and preservative already present. Daily inversion of the composite bottle or a gentle swirling of its contents reduced the bacterial count to a low figure (under 10000/ml). High mould counts were probably due to contamination from the bottle and stopper since the subsequent use of sterilized bottles and stoppers practically eliminated this form of infection.

The use of preservatives other than mercuric chloride

Since other preservatives probably have no stimulating effect on lipase (Manus & Bendixen, 1959), several of the better known ones were tested under practical conditions. There was little or no increase in free fat acidity with potassium dichromate, but this appeared to be less reliable than mercuric chloride in preserving milk at ambient temperatures, and it also tended to promote fat oxidation. Formaldehyde solution (40 %, w/v) and solid paraformaldehyde tablets were satisfactory not only as preservatives but in controlling fat acidity. Unfortunately, the difficulty in dissolving the 'hardened' casein in sulphuric acid is a disadvantage in factory testing. Neither phenyl mercuric acetate nor hydrogen peroxide was effective in controlling bacterial action in 10-day composite samples.

Several commercial quaternary ammonium compounds, some with emulsifying properties, were also investigated. All those tested precipitated the milk protein either at once or on standing overnight. Since these compounds were alkaline in reaction it appeared that protein denaturation preceded precipitation.

A number of antibiotics, including the 'broad spectrum' type such as aureomycin or terramycin, or a mixture of both, were ineffective in preserving milks for more than 5 days. The appearance of some milks suggested that decomposition was due to fungi and not to bacteria.

DISCUSSION

In many composites sampled at factory A at 85–90 °F the fat was obviously not dispersed evenly throughout the milk. At factory B, mixing at a higher temperature (98–103 °F), ensured that fat clumps were broken up and that the milk drained cleanly from the pipette. Even at this temperature, however, the results were often low presumably because of churned fat or of free fat. Thus, with many factory samples uniform dispersion of fat was possible only after high-speed mixing at 115 °F. Signs of churning were more pronounced when samples of fresh milk had been brought long distances by tanker in warm weather. The use of better cooling on the farm and the transport of the samples in insulated iceboxes on the tanker would probably overcome this problem.

The high free fat acidities (up to 25) reported by Manus & Bendixen (1956) were obtained when their composites were held at 35–40 °F so that fat hydrolysis presumably was due to the activity of natural lipase. In the present work, the factory composites were held at 50–80 °F and bacteria and moulds were often present, so that the increase in fat acidity could have been due either to natural lipase or to microbial lipases. Of the preservatives tested only mercuric chloride had a stimulating effect on fat hydrolysis. Since a number of the factory composites, all of the laboratory composites, and many of the experimental dairy composites showed little or no increase in fat acidity over 10 days, it appears that, even in the presence of mercuric chloride, fat hydrolysis occurs only under certain conditions.

Increases in free fat acidity to values as high as 10 or even 20 apparently had little effect on the Babcock result, although Manus & Bendixen (1956, 1959) reported reductions of 0·05–0·15 % fat over this range. Larger increases in fat acidity, as occurred in some samples held after factory testing, would probably lower the Babcock result appreciably.

The increase in Gerber results with increasing fat acidity could be explained by the presence of esters of amyl alcohol and lower fatty acids in the fat column (Fort, 1949). Butyric acid from lactose fermentation can also raise the Gerber result (Day, 1920). The difference of 0·059% between the average Gerber result and the average Werner Schmid result for all the factory composites could have been due to: (*a*) the error introduced by use of a 10·94-ml pipette for milks with fat percentages higher than 3·80 (British Standards Institution, 1955); and (*b*) esters in the fat column in milks high in fat acidity or in bacterial count.

The slight decrease in the Werner Schmid and the Babcock results for 8 laboratory composites compared with the averages for all the daily milks (see Table 4) is not easily explained since there had been no increase in fat acidity and the fat had been dispersed by high-speed mixing. It is unlikely that the diluting effect of the mercuric chloride tablets would reduce the fat content by more than 0·005%. The composite results were sufficiently close to the average for the daily results, however, to indicate that both Babcock and Gerber tests will give reliable results on composite samples properly handled and kept at temperatures preferably in the range 50–60 °F. The experimental dairy composites kept at 38–40 °F were all in excellent condition but varied quite widely in fat acidity. High fat acidity in these milks, however, did not cause the Babcock test results to be any lower than they were for milks of low free fat acidity.

Despite the possibility of fat hydrolysis, mercuric chloride still appears to be the most reliable preservative for composite milk samples required for fat testing. Provided that the fresh milk samples arrive at the factory in good condition, that the composite bottles and stoppers are sterilized, that the milk and preservative are properly mixed, and that the composite is held at 55–60 °F and mixed at 98–103 °F before sampling, then reasonably accurate results may be expected. If, however, the daily samples are in poor condition and the composites show signs of churned or free fat, then only mechanical mixing at 115 °F will ensure that a representative sample is obtained for fat testing.

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Fat testing of composite Milk samples with the Milko-tester

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Summary. With 10-day composite milk samples the effect has been investigated of various preservatives, of variations in the method of preparing the composites and of the condition of the composites at sampling on Milko-tester results for fat content.

The results for composites preserved with mercuric chloride agreed much more closely with the Werner Schmid results than did those for corresponding composites preserved with potassium dichromate, formaldehyde solution or paraformaldehyde.

In composites preserved with mercuric chloride, prepared and held at ambient temperatures at 2 dairy factories, a small proportion contained destabilized fat when sampled at 98–103 °F and gave very low Milko-tester results in comparison with the Werner Schmid and Gerber results. After high-speed mixing of the composites at 115 °F the Milko-tester results were closer to, but still lower than, the Werner Schmid results.

The average Milko-tester result for 133 composites free from destabilized fat when sampled at 98–103 °F was $0{\cdot}099 \pm 0{\cdot}055$% lower than the average Werner Schmid result. Results for 133 duplicate samples, which were also free of destabilized fat at 98–103 °F but which had not been swirled after the daily addition of fresh milk, were on average $-0{\cdot}158 \pm 0{\cdot}076$% lower than the Werner Schmid result. The Werner Schmid and Gerber results agreed well. The lowering of the Milko-tester result in the 'swirled daily' samples was due (*a*) to the effect produced by dissolving mercuric chloride in milk, (*b*) to slight fat hydrolysis, and (*c*) to some other factor, possibly fat oxidation. The further lowering of the Milko-tester result in the 'non-swirled' samples was due mainly to greater hydrolysis of the fat with production of higher fat acidities. Other composites, which were swirled daily but which were held at 38–40 °F gave on the average higher fat acidities and lower Milko-tester results than the 'swirled daily' composites held at ambient temperatures.

A correction of $+0{\cdot}10$% to the Milko-tester readings of all 'swirled daily' composites, preserved with mercuric chloride and in suitable condition for sampling at 98–103 °F, brought over 90% of the results to within $\pm 0{\cdot}10$% of the Werner Schmid results.

The Milko-tester (Foss Electric, Hillerød, Denmark) is already in use in a number of countries for the rapid estimation of fat in milk. The fat content is read directly by a photometric measurement of the turbidity due to the fat globules after heating, homogenizing and diluting the sample with a protein-dissolving versene solution. The instrument has been found satisfactory for the routine testing not only of fresh milks but also of milks to which preservative has been added (Černá & Písecký, 1966; Murphy & McGann, 1967). Grubb (1966), however, reported that Milko-tester read-

ings for samples preserved with potassium dichromate were lower after 14 days' storage.

In the present paper the results are presented of an investigation into the suitability of the Milko-tester for fat testing of 10-day composite milk samples.

EXPERIMENTAL

Fresh milk samples

A comparison of Gerber and Milko-tester results was made on several occasions through the 1966–7 dairy season on milks from individual cows from a herd of mixed (predominantly Jersey) breeds.

During the same season, Werner Schmid and Milko-tester results were compared for Jersey-herd samples selected at random from the farm samples brought in daily by tanker to a dairy factory.

Composite milk samples

(*a*) *Laboratory composites*

To compare the effect of several preservatives on Milko-tester results, 10-day composites were prepared in the laboratory by the daily addition of 15 ml of fresh milk. Each composite contained: (i) 2 tablets of mercuric chloride (0·13 g of mercuric chloride/tablet of 0·26 g), or (ii) 2 tablets of potassium dichromate (0·32 g), or (iii) 0·3 ml of formaldehyde solution (40%, w/v), or (iv) 0·15 g of paraformaldehyde. The composites were swirled each day after the addition of milk and kept at laboratory temperatures and unprotected from light. After 10 days, the samples were warmed to 98–103 °F and tested for fat by the Werner Schmid and Gerber methods and by the Milko-tester.

(*b*) *Factory composites*

Over the period November 1966–March 1967 a large number of 10-day composite samples were prepared for the Institute by the staff at 2 dairy factories. Each day, 2 dipperfuls (approximately 15 ml/dipperful) of fresh milk from a numbered ½-pint farm sample were added to each of 2 sterilized 1-pint bottles containing 4 tablets of mercuric chloride. One bottle, the 'non-swirled' sample, was replaced on the test room shelf with no swirling or mixing of the contents as is the usual procedure in many dairy factories. The other bottle, the 'swirled daily' sample, was gently swirled to mix the fresh milk thoroughly with the preserved milk already present. The samples were all kept at ambient temperature and were not protected from light.

At the end of the 10-day period the composites were brought to the Institute for analysis. Each sample was warmed to 98–103 °F, a small portion poured off for bacteriological analysis and a larger portion for further heating to 115 °F before mixing with a high-speed (Polytron) mixer. This latter portion was allowed to cool to about 80 °F before being tested for fat by the Werner Schmid method and by the Milko-tester. The remainder of the sample at 98–103 °F was also tested for fat both by the Gerber test and by the Milko-tester. A free fat acidity estimation was also carried out on this sample.

(*c*) *Market milk composites*

From December 1966 to May 1967 a number of composites were prepared for the Institute at a plant processing market milk. The daily (30 ml) sample of fresh milk for each composite was taken from $\frac{1}{2}$-pint samples brought in by tanker, in an insulated ice box from farms with refrigerated cooling. Each 1-pint composite bottle contained 2 tablets of mercuric chloride (i.e. only half the amount of preservative used in the factory samples), was kept at 38–40 °F for the whole of the 10-day period and was swirled each day to mix the contents. At the end of the 10-day period the milks were brought to the laboratory, prepared for analysis and analysed by the methods described for the factory samples.

Preserved milk with added lipase

The effect of large increases in free fat acidity on Milko-tester readings was investigated by the addition of 0·1 % of a commercial lipase product to milk preserved with mercuric chloride (1 tablet to 75 ml milk). Five 100-ml portions of the preserved 'lipase' milk and 5 of the preserved control milk were stored in small bottles in the laboratory and 1 bottle from each group examined after 0, 5, 7, 9 and 12 days for fat acidity and for fat content by the Milko-tester.

Fat estimations

(*a*) *Operation of Milko-tester*

A Milko-tester, Mark II, was operated according to the manufacturer's instructions except that fresh milk of known fat content (by Gerber test) was used each day, first to set the instrument and then as a check after each zero adjustment. Since readings for the control milk tended to decrease over the operating period, the sample readings following each check were corrected for the difference between the check reading and the original reading. Duplicate estimates were made on each sample. The modification suggested by Murphy & McGann (1967) of periodically adjusting the scale reading to the correct fat figure for the control milk without zero adjustment has recently been tried out in this laboratory and found to be less troublesome and more satisfactory than the method described.

(*b*) *Werner Schmid method*

Since fat hydrolysis may occur in composite samples at atmospheric temperatures (McDowell, 1968) and since in the Werner Schmid method a much greater proportion of the fatty acids are extracted than with the Röse–Gottlieb method, the former method (slightly modified—see McDowell, 1968) was used as a standard for fat estimation.

(*c*) *Gerber method*

Duplicate estimations were made (British Standards Institution, 1955) using butyrometers which were accurate to within 0·02 %.

Free fat acidity

Free fat acidity of milk samples was estimated by a method described previously (McDowell, 1964).

Bacteriological analysis

A plate count after incubation at 30 °C for 48 h on lactose yeast phosphate agar was made on each factory composite sample.

Previous work on composite samples (McDowell, 1968) had shown that moulds developed on this medium, often in large numbers. With samples in the present investigation, however, there was an almost entire absence of moulds presumably because the composite bottles and stoppers were sterilized before use.

RESULTS

Fresh milk samples

Results for 35 herd milks over the range 3·92–6·25% fat were on average 0·015 ± 0·053% lower when determined with the Milko-tester than when measured by the Werner Schmid method. For 69 milk samples from individual cows, with fat contents varying from 2·79 to 7·00%, results with the Milko-tester were 0·025 ± 0·073% lower than those measured by the Gerber method.

Table 1. *Effect of mercuric chloride and of potassium dichromate as preservatives on average Werner Schmid and Milko-tester results for laboratory composites*

		Mercuric chloride	Potassium dichromate
No. of samples		24	24
Werner Schmid fat, %		4·448	4·444
Milko-tester fat, %		4·369	4·242
Difference from Werner Schmid		−0·079	−0·202
Free fat acidity	Average	2·30	1·72
	Range	1·4–6·3	1·2–3·6

(*a*) *Laboratory composites: effect of different preservatives*

Preliminary tests showed that if tablets of either mercuric chloride or potassium dichromate were dissolved in fresh milk, 2 tablets in 150 ml of milk, the Milko-tester reading was immediately lowered by 0·02–0·05%.

The average Milko-tester result for composites preserved with mercuric chloride and sampled at 98–103 °F was 0·079% lower and for duplicate composites preserved with potassium dichromate was 0·202% lower than the Werner Schmid result (see Table 1). Results for composites preserved with pure mercuric chloride and unprotected from light or for composites preserved with either mercuric chloride tablets or potassium dichromate and kept in the dark at laboratory temperatures showed differences very similar to those above. In composites preserved with a mixture of both compounds in the proportion of 3 parts of mercuric chloride to 13 parts of potassium dichromate as used by Murphy & McGann (1967) the Milko-tester readings were lowered to the same extent as by an equal weight of dichromate alone.

In composites preserved with formaldehyde the Milko-tester readings, in contrast

to those with mercuric chloride or potassium dichromate, were higher than the Werner Schmid results by an average of 0·180% (see Table 2). Since paraformaldehyde had almost exactly the same effect, the results with this preservative are not quoted.

Table 2. *Effect of mercuric chloride and of formaldehyde solution as preservatives on average Werner Schmid and Milko-tester results for laboratory composites*

	Mercuric chloride	Formaldehyde solution
No. of samples	4	4
Werner Schmid fat, %	4·863	4·870
Milko-tester fat, %	4·796	5·050
Difference from Werner Schmid	−0·067	+0·180

Table 3. *Average Werner Schmid, Milko-tester and Gerber results for factory composites in good condition for sampling at* 98–103 °F

		'Non-swirled'	'Swirled daily'
No. of samples		133	133
Werner Schmid fat, %		4·807	4·805
Milko-tester fat, sampled at 98–103 °F, %		4·649	4·706
Difference from Werner Schmid		−0·158	−0·099
S.D. of difference		±0·076	±0·055
Milko-tester fat, sampled after high-speed mixing, %		4·656	4·719
Difference from Werner Schmid		−0·151	−0·086
S.D. of difference		±0·066	±0·064
Gerber fat, sampled at 98–103 °F, %		4·830	4·817
Difference from Werner Schmid		+0·023	+0·012
S.D. of difference		±0·048	±0·046
Free fat acidity	Average	4·58	2·77
	Range	1·1–12·5	1·2–7·4

(*b*) *Factory composites preserved with mercuric chloride*

The average results for 133 'non-swirled' composites and for a duplicate set of 133 'swirled' samples, all of which were in good condition for sampling at 98–103 °F, are shown in Table 3. Although the average Werner Schmid and Gerber results for both groups of samples were very similar, the Milko-tester results after high-speed mixing of the samples were lower than the Werner Schmid by 0·086% for the 'swirled daily' and by 0·151% for the 'non-swirled' samples. The differences were slightly greater for the Milko-tester results at 98–103 °F (Table 3). There were wide variations in the fat acidity figures, the average for the 'non-swirled' being considerably higher than that for the 'swirled daily' samples (Table 3). It was found also that many of the 'non-swirled' samples gave relatively high bacterial plate counts (up to 700000/ml), whereas most of the 'swirled daily' samples gave counts of less than 10000.

Not all of the factory composites received were in a suitable condition for sampling at 98–103 °F. Certain composites prepared from milk samples brought long distances by tanker in warm weather, with no proper cooling, showed signs of churned and free

fat at this temperature. The results for 16 of these composites (8 'non-swirled' and 8 duplicate 'swirled daily' samples) are shown in Table 4. For the 'non-swirled' composites the average Milko-tester reading after sampling at 98–103 °F was 0·118% below that obtained after high-speed mixing and for the 'swirled daily' composites it was 0·114% below. Even after high-speed mixing, however, the Milko-tester result for the 'non-swirled' composites was still 0·22% below the Werner Schmid result compared with 0·062% for the 'swirled daily' composites (see Table 4) because of the effect of high free fat acidity (see below). It will be noted, however, that the condition of the samples at 98–103 °F had little effect on the Gerber test since the average results were very close to the Werner Schmid results. The plate counts for the 'non-swirled' composites (100000–800000/ml) were again much higher than those for the swirled composites (nil to 15000/ml).

Table 4. *Average Werner Schmid, Milko-tester and Gerber results for factory composites containing destabilized fat when sampled at* 98–103 °F

	'Non-swirled'	'Swirled daily'
No. of samples	8	8
Werner Schmid fat, %	5·213	5·219
Milko-tester fat, sampled at 98–103 °F, %	4·875	5·043
Difference from Werner Schmid	−0·338	−0·176
Milko-tester fat (sampled after high-speed mixing), %	4·993	5·157
Difference from Werner Schmid	−0·220	−0·062
Gerber fat, sampled at 98–103 °F, %	5·223	5·221
Difference from Werner Schmid	+0·010	+0·002
Free fat acidity Average	7·59	3·25
Range	5·8–12·0	1·8–4·7

Table 5. *Effect of increases in free fat acidity on average Werner Schmid, Milko-tester and Gerber results for factory composites*

	'Non-swirled'	'Swirled daily'
No. of samples	33	33
Werner Schmid fat, %	4·828	4·823
Milko-tester fat, sampled at 98–103 °F, %	4·659	4·741
Difference from Werner Schmid	−0·169	−0·082
Milko-tester fat (sampled after high-speed mixing), %	5·678	4·758
Difference from Werner Schmid	−0·150	−0·065
Gerber fat, sampled at 98–103 °F, %	4·855	4·836
Difference from Werner Schmid	+0·027	+0·013
Free fat acidity Average	6·10	2·66
Range	5·0–8·5	1·8–3·8

High fat acidities in composites preserved with mercuric chloride had previously been found to increase the Gerber test and to lower the Babcock test (McDowell, 1968). Since in the present investigation many factory composites, especially the

'non-swirled' samples, showed increases in fat acidity the effect on Milko-tester readings was investigated. Table 5 shows average results for 33 'non-swirled' samples with fat acidities greater than 5·0 compared with results for 33 duplicate 'swirled daily' samples low in fat acidity. An average increase in fat acidity from 2·66 to 6·10 apparently caused an average decrease of 0·085% in Milko-tester readings. Further confirmation of the effect of fat acidity on the readings was obtained in an experiment in which added lipase (0·1%) was added to milk preserved with mercuric chloride. The results in Table 6 show a steady decline in Milko-tester readings as the fat acidity increased. The control milk with only slight changes in fat acidity also showed a slight drop in Milko-tester readings after 7 days but this could conceivably have been due to fat oxidation which, in other samples, was shown to occur in composites unprotected from light.

Table 6. *Effect of addition of lipase on Milko-tester results for milk preserved with mercuric chloride*

	Control			Added lipase		
Days stored	Free fat acidity	Fat, %	Difference from fresh milk	Free fat acidity	Fat, %	Difference from fresh milk
0	1·6	5·16	—	1·6	5·16	—
5	1·9	5·16	0·00	16·5	4·86	−0·30
7	1·6	5·11	−0·05	18·8	4·77	−0·39
9	2·3	5·07	−0·09	21·7	4·60	−0·56
12	2·2	5·06	−0·10	25·2	4·38	−0·78

Table 7. *Average Werner Schmid, Milko-tester and Gerber results for market milk composite*

No. of samples	72
Werner Schmid fat, %	4·154
Milko-tester fat, sampled at 99–103 °F, %	4·053
Difference from Werner Schmid	−0·101
S.D. of difference	±0·063
Milko-tester fat, sampled after high-speed mixing, %	4·050
Difference from Werner Schmid	−0·104
S.D. of difference	±0·066
Gerber fat, sampled at 98–103 °F, %	4·173
Difference from Werner Schmid	+0·019
S.D. of difference	±0·037
Free fat acidity Average	4·16
Range	1·6–14·2

(c) *Market milk composites preserved with mercuric chloride*

Average results for 72 market milk composites kept at 38–40 °F and swirled each day are shown in Table 7. The Milko-tester result was 0·10% below the Werner Schmid result, both after sampling at 98–103 °F and after high-speed mixing.

High fat acidities in some of the market milk composites again appeared to affect the Milko-tester readings. Thus, for 7 milks with an average fat acidity of 7·4 the

average Milko-tester reading was 0·175% below the Werner Schmid reading, whereas for 7 other milks drawn from the same batches of samples but with an average acidity of 2·2, the instrument reading was only 0·086% lower.

(d) Correction of Milko-tester readings

A frequency distribution of the differences between the Milko-tester readings (after sampling at 98–103 °F) and the Werner Schmid results (after high-speed mixing) for the 133 'swirled daily' factory samples are shown in Table 8. The differences for the market milk samples are also shown in Table 8. If all the Milko-tester readings were increased by 0·10% then 97% of the factory samples and 91·7% of the market milk samples would show a difference not greater than ±0·10% from the Werner Schmid result.

Table 8. *Frequency distribution of the differences between the Milko-tester results and the Werner Schmid results for factory 'swirled daily' composites and for market milk composites*

Description of composites	No. of milks	Werner Schmid fat percentage minus Milko-tester fat percentage: Range of differences	No. of samples	% of total number of samples
Factory 'swirled daily'	133	+0·21 to +0·30	3	2·3
		+0·11 to +0·20	59	44·4
		+0·06 to +0·10	39	29·3
		+0·01 to +0·05	27	20·3
		0·00	4	3·0
		−0·01 to −0·05	1	0·7
Market milk	72	+0·21 to +0·30	6	8·3
		+0·11 to +0·20	22	30·6
		+0·06 to +0·10	24	33·3
		+0·01 to +0·05	20	27·8

DISCUSSION

Results for fresh milks indicate that the accuracy of the Milko-tester was similar to that claimed by the manufacturers and to that obtained by other investigators (Brems, 1966; Černá & Písecký, 1966; Hänni, 1966; Murphy & McGann, 1967; Schmidt, 1966; Senft, Grochowalski & Cieslar, 1965).

Both mercuric chloride and potassium dichromate dissolved in fresh milk appeared however, to lower the Milko-tester reading to a much greater extent than would be anticipated from the diluting effect of the preservatives themselves. In 10-day composite milks the greater drop in instrument readings with potassium dichromate than with mercuric chloride was not due to fat hydrolysis (see Table 1), but could have been due to fat oxidation since the samples were tallowy and the fat gave high peroxide values. Milk preserved with mercuric chloride, however, also gave evidence of fat oxidation. High Milko-tester readings with formaldehyde solution or paraformaldehyde as preservative could have been due to the 'hardening effect' of these preservatives on casein, thus causing a lowered protein solubility in fersene solution. Since the difference between the Milko-tester readings and the Werner Schmid results was less with mercuric chloride than with the other preservatives, and since

it had other advantages as a preservative (McDowell, 1968) its use in further trials with the Milko-tester appeared to be justified.

The later trials showed very clearly that the condition of the composite samples when warmed to 98–103 °F had a greater effect on the Milko-tester result than on that from conventional methods. Apparently any destabilized fat tended to rise to the surface of the milk in the bottle during transfer of the sample to the Milko-tester. High-speed mixing of these samples was an essential preliminary to accurate analysis.

Even moderate increases in fat acidity reduced the Milko-tester results quite appreciably. Thus with all composites at atmospheric temperatures, proper daily mixing was necessary to prevent bacterial growth and to reduce hydrolysis of the fat by microbial or natural lipase (McDowell, 1968).

Some market milk composites, although in excellent condition for sampling at 98–103 °F, gave low Milko-tester readings because of high fat acidities. The effect of holding at 38–40 °F on hydrolysis of milks preserved with mercuric chloride has been noted previously (Manus & Bendixen 1956, 1959; McDowell, 1968). Since average increases in fat acidity were smaller at atmospheric temperatures than at 38–40 °F, composite samples might well be kept at 55–60 °F rather than at refrigeration temperatures (McDowell, 1968).

A correction of +0·10 % to the Milko-tester readings for the 'swirled daily' factory and market milk composites would bring over 90 % of the results within 0·10 % of the Werner Schmid results. Thus in practice, if the Milko-tester reading for the control (fresh) milk were set 0·10 % higher than the Gerber test result then most of the instrument readings for composite milks which had been swirled each day and were in good condition for sampling at 98–103 °F would be reasonably correct.

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Biuret and urea in concentrates for milking cows

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Summary. Two experiments, one with 18 and one with 21 cows, were made to compare the feeding value for milk production of concentrates in which the nitrogen normally supplied by oil cake was completely replaced by either biuret or urea. In expt 1 the urea and biuret contributed 52% of the total nitrogen in the concentrate and in expt 2 they contributed 43%. Hay was fed as the sole roughage. The estimated energy intake in expt 1 was 102–104% of the Woodman standard and in expt 2 it was 108–109%.

In both experiments milk production was about 10% less on the urea and biuret treatments than on the control treatment. There was no significant difference in milk yield between the biuret and the urea treatments. The percentages of fat and protein in the milk from the urea and biuret treatments were higher than from the control treatment, but the weights of these milk constituents were slightly lower than from the control treatment. Liveweight changes and water consumption were not affected by the experimental treatments. In expt 2 an intake of nitrogen about 20% lower than is often recommended in the United Kingdom did not appear to depress milk yields. Three cows that had received biuret concentrates in expt 2 continued to do so during a nitrogen balance trial lasting 3 weeks. Each cow was slightly in negative balance, the average value being −4 g N per cow per day.

It has been known for many years that urea can be used as a source of nitrogen in the rations of milking cows (Owen, Smith & Wright, 1943; Reid, 1953; Armstrong & Trinder, 1966). In general, the results indicate that urea N is utilized by the cow less efficiently than is the N in such material as oil cake, and this has been ascribed partly to the too rapid release of ammonia in the rumen as the result of enzyme action, although a recent report suggests that urea feeding leads to a lower concentration of some essential amino acids in the plasma (Oltjen & Putnam, 1966). Urea, in the amounts used for feeding dairy cows, is completely soluble in rumen liquor and it would appear that if an NPN source of lower solubility were used, then the evolution of ammonia might be slower, and its utilization more efficient. Conversion of urea to biuret gives a compound which has a solubility of about 2·2% in rumen liquor at 39 °C, and it has been reported by Hatfield, Garrigus, Forbes, Neumann & Gaither (1959) to be non-toxic to ruminants and to be a suitable source of nitrogen for growth in lambs and calves and for reproduction in sheep. Since then, however, conflicting reports on its usefulness have appeared, but according to Schaadt, Johnson & McClure (1966) this variability may have arisen from a com-

parison of results obtained from *in vitro* experiments with those from *in vivo* work. The present paper records the use of biuret, at 2 levels, as a replacement for all the nitrogen normally derived from oil cake in the concentrate part of the ration of milking cows. Concentrates containing oil cake were used as controls and concentrates containing urea were included for comparison of the NPN source. Two feeding experiments with milking cows were made, and N retention was measured in 3 of the cows receiving the biuret concentrate.

EXPT 1 (1965–6)

Experimental

The design of expt 1 was of the balanced change-over type with 18 Ayrshire cows. The animals were divided into 6 groups with the cows in each group as similar as possible in milk yield and stage of lactation. They were then allotted at random to the treatment sequences in 2 orthogonal 3 × 3 Latin squares. The average lactation number of the cows was 3, with only 4 of them having had more than 3 lactations. Each cow had been milking for 60–70 days before the experiment started.

Table 1. *The ingredients of the concentrate mixtures*

	Composition, % on air-dry basis		
	Control	Biuret	Urea
Ingredient	% on air-dry basis		
Expt 1			
Biuret*	—	3·9	—
Urea*	—	—	2·9
Decorticated groundnut cake	23·3	—	—
Barley	66·6	81·9	85·7
Starch	—	7·0	4·2
Oat husks	2·9	—	—
Minerals*	2·4	2·4	2·4
Molasses	4·8	4·8	4·8
Expt 2			
Biuret*	—	2·6	—
Urea*	—	—	2·1
Soyabean meal	2·5	—	—
Decorticated groundnut cake	5·8	—	—
Decorticated cotton cake	2·5	—	—
Linseed cake	3·5	—	—
Copra cake	10·7	—	—
Barley	33·0	43·7	44·0
Maize	33·0	43·7	43·9
Starch	—	2·0	2·0
Minerals†	3·0	3·0	3·0
Molasses	5·0	5·0	5·0

* Biuret; 7 % moisture, 39 % N in D.M. Urea; 0·7 % moisture, 46 % N in D.M.

† Supplements of vitamins A and D_3, trace minerals and spices were added to all concentrates, and in addition elemental sulphur (520 g/ton) was added to the biuret and urea concentrates.

Each diet consisted of good quality hay with one of 3 concentrate mixtures and was fed for periods of 7 weeks. The formulation of the 3 concentrates is given in Table 1, from which it can be seen that the biuret and urea N replaced that derived

from groundnut cake in the control concentrate. The biuret and the urea both supplied 52% of the total N in these concentrates. Barley was the main source of energy and was supplemented by potato starch in the biuret and urea concentrates. In period 1 of the experiment, when milk yields were highest, 4 lb of concentrates were fed at midday, and the remainder was divided equally and fed during the morning and evening milkings. In periods 2 and 3, the concentrates were divided equally and fed at the morning and evening milkings. The concentrates were fed at the rate of 4 lb/10 lb milk, and the weight fed in period 1 was calculated from the mean daily weight of milk in the week preceding the experiment. In periods 2 and 3, the amounts fed were based on the equalized feeding principle of Lucas (1943) using the average daily milk yield of the last week of the previous period and the average decline in milk yield of the group. Any refusals of hay or concentrates were weighed and the dry matter (D.M.) of feeds and refusals determined on 5 days of each week.

Table 2. *Analysis of the feedingstuffs*

		Percentage of the D.M						
	D.M., %	Total N	NPN	Crude fibre	Ether extract	Ash	Lignin	Total soluble sugars
Expt 1								
Control concentrate	83·6	3·17	0·25	6·3	3·3	5·2	3·1	ND*
Biuret concentrate	83·0	3·15	1·84	6·1	2·0	5·1	2·4	ND
Urea concentrate	83·2	3·16	1·82	5·4	1·8	5·0	2·6	ND
Hay	81·4	1·46	0·42	31·3	2·0	6·3	8·2	9·2
Expt 2								
Control concentrate	85·5	2·75	0·32	5·7	4·2	7·7	2·7	ND*
Biuret concentrate	85·1	2·55	1·24	3·8	3·4	5·5	1·8	ND
Urea concentrate	85·0	2·60	1·27	3·6	3·2	5·4	1·9	ND
Flaked maize	83·6	1·52	0·34	3·0	2·9	0·9	1·4	ND
Hay	80·7	1·60	0·51	30·4	2·4	6·0	9·4	9·7

* ND = not determined.

The analyses of the 3 concentrates and of the hay are given in Table 2. A total of 17 lb of hay was fed per day, made up of 6 lb in the morning, 3 lb at noon and 8 lb at night. The average energy value of the hay, expressed on a D.M. basis, was calculated from the crude fibre percentage and the regression equations of Alderman, Collins, Jones, Morgan & Ibbotson (1967), and was 41 S.E. or 0·98 Mcal metabolizable energy/lb. The average digestible crude protein content was 4·6%. The calculated energy value of each of the concentrates was 80 S.E. (Evans, 1960) or 1·33 Mcal metabolizable energy/lb (Agricultural Research Council, 1965), on a D.M. basis. It can be seen from Table 2 that the 3 concentrates had similar total N contents and for the control concentrate this was equivalent to 19·8% crude protein, with a calculated digestible crude protein content of 16·7% in the D.M. The apparent digestibilities of the D.M. and of the N of the diets used in the 3 feeding treatments, as measured with 3 other milking cows, were found to be similar, 67–69% for D.M. and 66–67% for nitrogen.

On 2 separate days each week in the final 2 weeks of each feeding period the cows were milked with a quarter milking bucket. Samples of milk from each quarter were

taken for chemical analysis and for cell count as described by, respectively, Waite, White & Robertson (1956) and Blackburn, Laing & Malcolm (1955); in each of the other weeks, one 24-h whole-udder sample was taken for analysis. Urea-plus-ammonia N in the milk was determined after incubating a buffered, protein-free milk filtrate with melon seed meal for 2 h at 40 °C (Peters & Van Slyke, 1932). Ammonia was determined by aeration from an unincubated portion of the filtrate, and urea N calculated as the difference between the 2 values.

The cows were weighed at the same time on 2 consecutive mornings of each week.

Results

The control concentrate was palatable and there were no refusals. The biuret concentrate was slightly unpalatable to 2 of the 18 cows and this resulted in small amounts of food remaining uneaten, mainly in the first and second week of period 1 when yields were highest. The urea concentrate was not relished by any of the cows and small amounts of ground locust bean meal were added to increase palatability. Even so, 12 cows left small residues fairly regularly. The faeces from cows eating the biuret concentrate were softer than when they were eating the control or urea concentrates and, when analysed during the digestibility trial, the faeces arising from the biuret concentrate were found to average 13·8% D.M. compared with 15·4 and 14·7%

Table 3. *Expt* 1. *Dry-matter intake, milk yield and liveweight change*

	D.M. intake, lb per cow per day			Total intake as % of live-weight	Milk yield		Live-weight change, lb per cow per day
Concentrate	Hay	Concen-trates	Total		lb per cow per day	lb FCM per cow per day	
Control	13·7	9·7	23·4	2·43	25·5	25·7	−0·5
Biuret	13·6	9·5	23·1	2·40	23·0	24·5	−0·6
Urea	13·4	9·0	22·4	2·32	22·8	24·3	−0·5
S.E. of a difference between 2 means	±0·12	±0·10	±0·20	±0·016	±0·42	±0·49	±0·11

from the control and urea concentrates, respectively. The average daily feed intake and milk yield during the last 2 weeks of each period and the liveweight changes of the cows are given in Table 3. The biuret and the urea each contributed 31% of the total N of the average daily feed intake. The feed intake and milk production may appear somewhat low, but 11 of the 18 cows were in their first or second lactations and the length of the experiment (21 weeks) carried most cows into the last third of their lactations.

The energy intake (Table 4) was, by calculation, just about adequate according to the suggested requirements of both Woodman (Evans, 1960) and of the Agricultural Research Council (1965), but a loss of liveweight (Table 3) occurred on all 3 diets. Although such losses are often recorded for cows at this stage of lactation during the winter months, the losses measured may indicate some degree of underfeeding. The estimated intake of digestible crude protein was only 92% of that suggested by Woodman, but 120% of that suggested by the Agricultural Research Council.

There was no significant difference between the milk yields on the biuret and the

urea concentrates, either as recorded or on the basis of 4% fat corrected milk (Gaines, 1928), and both were significantly lower ($P < 0{\cdot}01$) than the yield on the control treatment. The weighted average composition of the milk produced from quarters of the udders free from subclinical mastitis is given in Table 5. Clinical mastitis occurred in 3 quarters and subclinical mastitis in 11 quarters during the experiment and inclusion of milk analyses from affected quarters lowered the average

Table 4. *Estimated nutrient intake per cow per day*

	S.E.		Metabolizable energy		Digestible crude protein		
Concentrate	lb/day	% of standard*	Mcal/day	% of standard†	lb/day	% of standard*	% of standard†
Expt 1							
Control	13·4	104	25·7	95	2·11	92	116
Biuret	13·2	102	25·0	101	2·07	92	120
Urea	12·7	102	24·2	99	2·03	92	120
Expt 2							
Control	16·0	109	30·8	100	2·11	78	100
Biuret	15·4	109	29·6	100	2·01	78	100
Urea	15·1	108	29·2	102	1·99	78	102

* Woodman (Evans, 1960). † Agricultural Research Council (1965).

Table 5. *Chemical composition of the milk*

	Percentage						mg/100 g			
Concentrate	Total solids	Fat	SNF	Lactose	Crude protein	Casein	Casein number	NPN	Urea N	Ammonia N
Expt 1										
Control	12·52	4·05	8·47	4·61	3·10	2·45	79·1	25·1	10·7	0·3
Biuret	12·96	4·45	8·51	4·61	3·16	2·48	78·3	25·9	8·7	0·3
Urea	12·86	4·37	8·49	4·55	3·19	2·48	78·6	24·9	9·7	0·4
S.E. of difference between 2 means	±0·07	±0·11	±0·02	±0·03	±0·03	±0·02	±0·20	±0·38	±0·72	±0·05
Expt 2										
Control	12·55	3·90	8·65	4·74	3·10	2·46	79·6	21·7	9·3	0·2
Biuret	12·61	4·04	8·57	4·60	3·15	2·49	79·1	22·5	8·7	0·2
Urea	12·73	4·14	8·59	4·62	3·15	2·48	78·5	23·2	9·3	0·3
S.E. of difference between 2 means	±0·28	±0·14	±0·06	±0·09	±0·12	±0·05	±1·08	±1·04	±0·70	±0·10

milk composition on all 3 treatments by about 0·5% solids-not-fat (SNF), 0·5% lactose and 0·4% in casein number. The fat percentage of the milk on the biuret and urea treatments was substantially and significantly higher than that on the control treatment, but, because of the lower milk yields on the urea and biuret treatments, the weights of fat produced on the 3 treatments were very similar, 1·03, 1·02 and 1·00 lb fat/day for the control, biuret and urea treatments, respectively. The percentage of protein in the milks on the biuret and urea treatments was slightly higher than that with the control concentrate, the difference reaching significance between the control and the urea treatments, but the weights of protein produced on the NPN treatments were lower than on the control treatment. Urea N tended to be

lowest in the milk from cows on the biuret treatment, but urea feeding did not lead to any significant rise in the amount of urea or ammonia in the milk. Milk samples taken during weeks 1–5 of each period showed that no violent fluctuations took place in the chemical composition of the milk on changing from one diet to another.

EXPT 2 (1966–7)

Experimental

A continuous-type design was used in expt 2 to examine the effect of more prolonged treatment, and in order to minimize the effects of individual animal variation with the small number of cows (21), a preliminary feeding period of 4 weeks was given during which all the cows received a high-quality commercial concentrate and the same hay that was to be fed during the experimental period. The results obtained in this preliminary period were later used to adjust the results from the experimental period by covariance analysis (Snedecor, 1956).

Twenty-one Ayrshire cows, calved from 30 to 80 days at the start of the preliminary feeding period, were used. These animals were grouped in 3s, with the cows in each group being balanced for stage of lactation, number of lactations and milk production in earlier lactations. The experimental feeding treatments, i.e. control, biuret or urea concentrates, were then randomly allocated to the 3 cows in each group. The average number of lactations/cow was 3.

In order to make the total amount of N supplied in the diets more critical in this experiment than in expt 1, the concentration of urea was reduced from 2·9 to 2·1%, and of biuret from 3·9 to 2·6%. This change lowered the contribution of the 2 NPN sources to 43% of the total concentrate N, compared with 52% in expt 1. The total N content of the concentrates in expt 2 was about 80% of that fed in expt 1. The composition of the concentrates is given in Table 1, and it can be seen that 5 different oil cakes were used, compared with only groundnut in expt 1. Barley and maize in equal parts replaced the barley used in the previous experiment. These changes were made to provide a control concentrate with a less soluble form of protein and one which was less dependent on the N content of a single cereal. Because of the high milk production of the cows during the preliminary feeding period, it was calculated that the 3 experimental concentrates would not supply sufficient energy for the higher yielding cows and, rather than increase the weight of concentrate fed, with a concomitant rise in the amount of N provided, a small amount of flaked maize (Table 6) was given at the same time as the concentrates. This maize was partly an addition and partly in substitution for the concentrates, and increased the energy intake but maintained the required N intake.

Sufficient concentrates were offered to supply the Agricultural Research Council (1965) estimated requirements for N and energy, and the rate of feeding was about 4 lb concentrate/10 lb milk with 16 lb good quality hay per day per 1000 lb liveweight. The rations were fed as in expt 1, but with the weight of concentrates being determined from the milk production 2 weeks earlier. Average figures for the chemical composition of the feedingstuffs are given in Table 2 and show the lower N content of the concentrates. The control concentrate contained 17·2% crude protein in the D.M., with a calculated digestible crude protein content of 13·8%. The hay had an

average crude protein content of 10·0% and an estimated digestible crude protein (D.C.P.) of 5% in the D.M. The average energy value of this hay was calculated to be 42 S.E. and 0·99 Mcal metabolizable energy/lb, and for the concentrates the corresponding values were 82 S.E. and 1·36 Mcal metabolizable energy/lb on a D.M. basis. The apparent digestibilities of the dry matter and of the nitrogen of the total diet of 3 cows receiving the biuret concentrates averaged 68 and 60%, respectively.

Milk samples from the whole udder were taken at 4 consecutive milkings once a week and the 4 samples pooled in proportion to yield, for chemical analysis. Cell count was measured weekly on the pooled sample and monthly on quarter samples from each cow.

Results

All the concentrates were palatable and there were no refusals. Except for occasional small residues of hay, mainly with 2 of the smaller cows, the hay ration was also consumed completely. The intake of D.M. from the feeds is given in Table 6,

Table 6. *Expt* 2. *Dry matter intake, milk yield and liveweight change*

	D.M. intake, lb per cow per day				Total intake as % live-weight	Milk yield, lb per cow per day		Live-weight change, lb per cow per day
Concentrate	Hay	Concentrate	Flaked maize	Total		Unadjusted	Adjusted	
Control	13·3	10·2	2·2	25·7	2·60	32·6	33·1	−0·1
Biuret	12·9	10·9	1·2	25·0	2·49	30·4	29·7	−0·3
Urea	13·2	10·0	1·5	24·7	2·45	29·2	29·3	−0·3
S.E. of a difference	±0·30	±0·63	±0·36	±0·96	±0·11	±2·3	±1·3	±0·15

together with liveweight change and the daily milk yield/cow averaged over the 15 weeks of the experiment. There were no statistically significant differences in the intakes of hay, concentrate or total D.M. with the 3 diets. Liveweight losses were higher on the NPN treatments than on the control treatment, but the differences were not statistically significant either before or after adjustment by covariance analysis. Total feed intake, expressed as a percentage of liveweight, was a little higher in this experiment than in expt 1 and the liveweight losses were less. The estimated nutrient intake is shown in Table 4 and these amounts are also given as percentages of the Woodman standard (Evans, 1960) and the Agricultural Research Council (1965) suggested requirements. The feeding régime was designed to fit the latter requirements and resulted in an energy intake that was above, and a nitrogen intake that was below, the Woodman standards.

The milk yields are given in Table 6 both as unadjusted values and after adjustment by covariance analysis. There was no significant difference between the unadjusted milk yields on the different treatments, but after adjustment the milk yield on the control treatment was significantly higher ($P < 0·05$) than those on the biuret and the urea treatments. There was no significant difference in milk production between the urea and biuret treatments. The course of milk production, however, differed somewhat on the 3 concentrate treatments, and Fig. 1 shows the average daily yield/cow from the 3 groups for each week of the experiment.

During the changeover week the average loss of milk by the 3 groups was control,

2·0 lb/cow; biuret, 2·0 lb/cow and urea, 2·7 lb/cow. In the first experimental week the control group cows increased their yields by 1·1 lb milk/cow, whereas the biuret group lost a further 3·3 lb/cow and the urea group lost a further 1·1 lb/cow. Thereafter, milk production of the cows on the control treatment declined steadily with advancing lactation, whereas that of the cows on both the urea and biuret treatments fell more rapidly during the first 5 weeks. This was particularly marked with the biuret treatment but after 5 weeks, milk production on this diet declined more slowly than did that with the other 2 treatments.

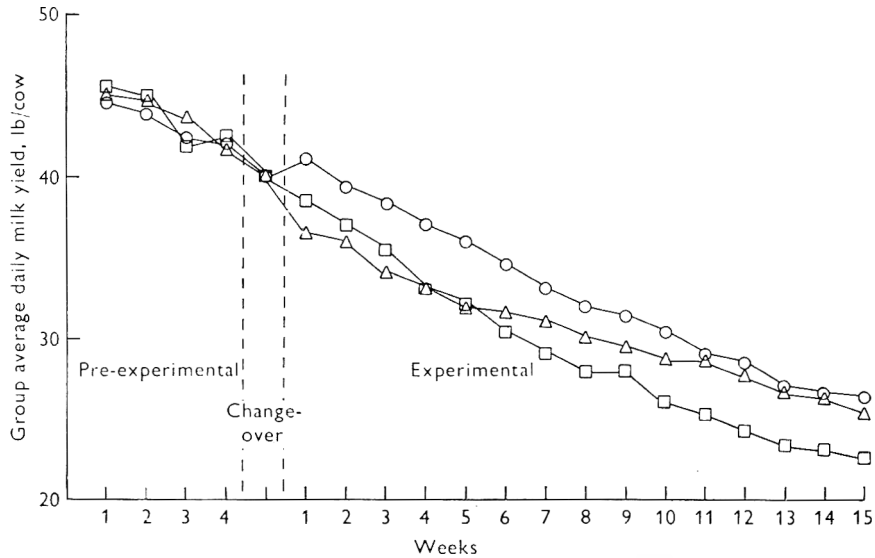

Fig. 1. The course of milk production by the 3 groups. ○, control; △, biuret; □, urea.

The average composition of the milk for the 3 treatments is given in Table 5. There was no significance between treatment differences in the percentages of the major constituents, either before or after adjustment. As in expt 1, the percentages of fat and protein were slightly higher in the milk of the cows receiving biuret and urea but, because of the lower milk yields on these treatments, the weights of fat and protein produced were less than were those from the cows on the control treatment. The tendency was again evident for the urea N in the milk of cows on the biuret treatment to be slightly lower than that in the milk from the other 2 treatments. The high lactose percentages and casein numbers indicated the absence of subclinical mastitis in all the cows and this was confirmed by the extremely low cell counts. These counts showed no significant difference between treatments and the 315 milk samples averaged only 60000 cells/ml. Only from 2 cows did the milk average more than 100000 cells/ml.

Water consumption

During expt 2 the water consumption of 6 cows, 2 on each feeding treatment, was measured daily and the maximum and minimum air temperatures recorded. The average temperature over the 15 weeks was 9·8 °C and lay within the range 9·5–11·5 °C

except for 2 weeks when it fell to 8 °C. The water requirements of the cows were calculated from the total water consumption, i.e. water drunk plus water eaten in the feeds, with a deduction for the water in the milk produced (0·87 × milk yield). The values for the control, biuret and urea treatments were 3·3, 3·3 and 3·5 kg water/kg D.M. consumed, which are close to the value of 3·5 kg water/kg D.M. eaten suggested by the Agricultural Research Council (1965) as being the requirement for a non-lactating cow.

The regressions of water consumption on D.M. intake and on milk yield were linear and analysis of variance showed that there was no significant treatment effect. From the pooled results the 2 regressions were:

$y = 5{\cdot}5 + 3{\cdot}99x$ where y = kg total water intake/day,
x = kg D.M./day (S.E. of coefficient = $\pm 0{\cdot}12$, $r = 0{\cdot}863$***),

and

$y = 27{\cdot}0 + 1{\cdot}75x$ where y = kg total water intake/day,
x = kg milk/day (S.E. of coefficient = $\pm 0{\cdot}12$, $r = 0{\cdot}855$***).

Nitrogen balance during biuret feeding

At the close of expt 2, three of the cows that had received the biuret concentrates for 15 weeks continued on the same diet for a further 3 weeks to allow a measurement of the nitrogen balances to be made. The collection and separation system described by Balch, Bartlett & Johnson (1951) was employed. Faeces were weighed at 8-h intervals and bulked for sampling and analysis each 48 h. Urine, preserved with 50 ml of 10% thymol in isopropyl alcohol, was weighed each 24 h and sampled for analysis. Milk was weighed and sampled at each milking and a weighted, pooled sample analysed each 48 h.

Table 7. *Nitrogen balance for* 3 *cows receiving biuret concentrates for* 18 *weeks, measured during the last* 3 *weeks, mean g N/day*

	N input			N output					Liveweight change in weeks 3–15, kg
Cow	Hay	Concentrate	Total	Urine	Faeces	Milk	Total	N Balance	
A	103·3	131·7	235·0	84·3	92·5	64·8	241·6	−6·6	−9·5
B	88·7	133·9	222·6	73·5	81·6	69·9	225·0	−2·4	−27·6
C	98·1	82·0	180·1	51·0	79·9	52·0	182·9	−2·8	+1·4

The results of the trial are given in Table 7 together with the liveweight changes during the last 12 weeks of the milking trial. (This period was chosen as providing a better estimate of the effect of diet on liveweight than the much shorter period of the balance trial). All 3 cows were in negative N balance, 2 of them to only a small extent but one, cow A, more definitely so. The average negative balance of −4 g N/day is the same as that found recently by Ulbrich, Wallmen & Mielke (1966) for 2 milking cows fed rations in which urea formed a similar proportion of the total N as did biuret in the present experiment. Assuming that the N balance figures as recorded in Table 7 for a 3-week period were typical of the whole 12-week period the liveweight change for cow A would be in rough agreement with the N balance and that for cow C would not be in serious disagreement, but there would be a greater

discrepancy in that of Cow B, a cow which lost weight steadily during the last 12 weeks of the milking trial. A consideration of all the results offers no clear explanation that would account for this marked fall in liveweight.

The proportion of total feed nitrogen appearing in the milk was 27·6, 31·4 and 28·9% for cows A. B and C. The corresponding values obtained by Owen *et al.* (1943) for 5 cows fed at a similar level of nitrogen with concentrates in which either blood meal or urea supplied most of the nitrogen averaged 28·5% for blood meal and 28·3% for urea. In the urine of cows A, B and C urea-N formed 62, 57 and 57% of the total nitrogen, compared with an average of 66% for 5 cows on the control treatment. The amount of biuret-reacting compounds in the urine of cows on all 3 treatments was very small but in the urine of the cows eating biuret it was about 40% greater than in the urine of cows on the control and urea treatments. Ammonia-N formed only 0·4–1·7% of the total urinary N of the cows on all 3 treatments.

DISCUSSION

The successful use of urea as a partial replacement for protein nitrogen in the feeding of milking cows was demonstrated many years ago, and more recently Virtanen (1966) has shown that cows consuming urea as the sole source of nitrogen milked normally throughout a whole lactation. The amount of urea that has been used in the past has been limited through fear of its toxicity and unpalatability, but Virtanen's work shows that if sufficient ammonium-converting bacteria are present in the rumen, as a result of preliminary slow adaptation, amounts as large as 6% urea in the concentrate, or 650 g urea/day, can be eaten by the cow without harming normal milk production. Whether cows receiving rations in which urea is only a partial replacement of the conventional dietary nitrogen can ever develop such an efficient ruminal flora is doubtful. The lower toxicity, lower solubility and higher palatability of biuret would appear to make it a better source of nitrogen. The results in this paper show that milk production was adequately maintained when either urea or biuret replaced conventional sources of vegetable protein in the ration, but that the amount of milk was somewhat less. This may have resulted from insufficient time for adaptation of the ruminal system to the biuret. Schaadt *et al.* (1966) found some evidence that lambs required 55 days for optimal use of biuret and similar or even longer periods have been suggested as necessary in urea feeding (McLaren, Anderson, Tsai & Barth, 1965; Virtanen, 1966). Thus, in expt 1, full adaptation of the rumen bacteria may have been accomplished only towards the end of each 7-week period, and milk yields may have been lower during the period of adaptation. In expt 2, the sharp initial rate of decline in the milk yield of cows receiving biuret was appreciably reduced after 5 weeks (Fig. 1), suggesting that more effective use was then being made of the biuret. A period of gradual adaptation to biuret, such as might have been provided during the pre-calving steaming-up period was precluded by the experimental design but could be readily achieved in good commercial practice.

In our experiments the lower solubility of the biuret did not lead to any improvement in milk production when biuret was compared with urea. Slightly less ammonia was present in the rumen liquor after feeding concentrates containing biuret than after feeding concentrates containing urea (Waite & Wilson, 1968), but the dif-

ference may have been insufficient to be reflected in increased milk production. The use of urea and biuret as sources of nitrogen gave rise to a higher fat percentage in the milk (significant in expt 1) and to slightly higher protein percentages. These advantages, however, were neutralized by the lower milk yields. The flavour of the milk produced on all treatments was normal throughout both experiments. The general health of the cows did not suffer and the use of biuret and urea did not lead to any increase in water consumption or urine excretion.

Based on the Woodman feeding standards (Evans, 1960) the supply of energy in both experiments was adequate, but the supply of digestible nitrogen was slightly below the recommended level in expt 1, and 22% below it in expt 2. Nevertheless, daily milk production declined at the rate of only 1 lb per cow per week in expt 2 which is a normal value for cows in winter milk production immediately after peak lactation. In the estimated energy requirements given by the Agricultural Research Council (1965) emphasis is laid on the concentration of energy in the total ration, i.e. M/D where M = total Mcal of metabolizable energy and D = total kg D.M. The average value for M/D in expt 1 was 2·4 and in expt 2 it was 2·6. Both these values may be slightly low for optimum milk production because of the relatively high intake of hay. However, the energy standards were approximately correct in both experiments although, as usual, such calculations are far from precise. In expt 1, the 'digestible nitrogen' intake was appreciably higher than the Agricultural Research Council (1965) estimate of requirement, but it was in agreement with it in expt 2. The results in expt 2, as judged by general level of milk production and rate of milk decline, support the conclusion arrived at some years ago by Wright (1939) that, as long as the cow is assured of an adequate supply of digestible energy, a lower intake of 'digestible nitrogen' than that suggested by Woodman does not appear to depress milk production. The results of the nitrogen balance experiment indicate, however, that at this lower level of nitrogen intake cows receiving biuret concentrates would probably be in slight negative balance at the end of the winter feeding period. Such a deficit could be quickly corrected during spring grazing.

The continuous type of trial would be clearly more suited to feeding experiments than some other designs were it not for the high coefficient of variation of the results when relatively few animals are used. Employing a 4-week pre-experimental feeding period and covariance analysis in expt 2, however, reduced the error term to approximately half its unadjusted value and indicated a significance in milk-yield differences that was absent in the unadjusted results. Moreover, continuous feeding allowed the adaptation period required in biuret feeding to be noticed, whereas in the Latin square design of expt 1 this was not possible.

The relatively small effect on milk production in our experiments when vegetable protein was partially replaced by urea and biuret is evidence of the utilization of the NPN by the rumen flora, but the other ingredients of the total ration still provided considerable quantities of vegetable protein. For example, using the average feed intakes shown in Tables 3 and 6, the biuret and urea each contributed only 31% of the total N of the ration in expt 1, and only 23% in expt 2. With the higher yielding cows in early lactation these values were 40 and 30% in expts 1 and 2, respectively. However, even 23% of the total N of the ration is an appreciable amount and if no biuret or urea had been supplied, reducing the total N of the diet to 60% of the

Woodman standard, a marked fall in milk yield and a decrease in the percentages of fat, protein and lactose might have been expected (Rowland, 1946), at least during the early weeks of the experimental period.

From these experiments it would appear that biuret can effectively replace the N usually supplied by oil cake in the concentrate part of the ration so long as the energy supply is adequate. If our conclusion that a period of adaptation is required by the rumen flora before maximum use is made of biuret N is correct, it could take place during pre-lactation feeding.

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The composition of rumen fluid from cows fed biuret and urea

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Summary. The effect on the chemical composition of rumen fluid of replacing oilcake nitrogen by either biuret or urea N in the concentrate part of the ration for cows has been investigated. The biuret treatment produced the highest concentrations of NPN and the lowest concentrations of ammonia N in the fluid, whereas the urea treatment gave rise to higher concentrations of NPN than did the oilcake treatment and the highest ammonia N concentrations of the 3 diets. It is unlikely, however, that the differences in either peak or mean ammonia concentrations between the biuret and urea diets, if they occurred in lactating cows receiving these diets, would be sufficient to affect milk production. The concentration of ammonia N in the rumen liquor of cows on the biuret diet increased progressively up to the 5th to 8th week of feeding this diet. The different dietary treatments did not result in any marked differences in the concentrations of total volatile fatty acids in the rumen fluids or in the proportions of acetic, propionic and butyric acids in the total fatty acids.

In a previous paper (Waite, Castle, Watson & Drysdale, 1968) 2 experiments were described in which dairy cows were given concentrates containing biuret or urea, the nitrogen from these compounds completely replacing that derived from oilcake in the control diet. The object of the experiments was to determine whether the relatively low solubility of biuret, which presumably might lead to a slower and more constant release of ammonia, would make it a more efficient source of nitrogen for milking cows than urea. To assist in the evaluation, the rumen fluid from 2 fistulated cows receiving the same diets as the milking cows was analysed. The present communication describes the effect of the type of concentrate on some constituents of the rumen fluid.

EXPERIMENTAL

Two 3-year old Ayrshire cows were each fitted with a rumen fistula; one cow, X, was producing milk at a low level during these investigations, the other cow, Y, was not in milk.

The detailed composition of the 3 concentrates has already been described (Waite *et al.* 1968). They had the same energy content and differed only in the major source of nitrogen. In the control concentrate this N was provided by oilcake and in the experimental concentrates by either biuret or urea. All the concentrates had the same total N content, biuret and urea providing 52% of it in the first year of the experiment (1965–6) and 43% of it in the second year (1966–7). Hay was fed as

the sole roughage and the ratio of hay to concentrates was the same for both cows in both years. The daily intakes of feed dry matter (D.M.), of total N and the amount of N contributed by biuret or urea are given in Table 1. Half of the daily ration was given at 6 a.m. and half at 4 p.m.

Table 1. *The daily intake of dry matter, total N and urea or biuret N*

Cow	Year	D.M. intake, kg		Total N, g	Urea or biuret N, g
		Hay	Concentrates		
X	1965–6	4·3	4·6	210	76
X	1966–7	4·3	4·6	177	52
Y	1965–6	3·6	3·9	174	62
Y	1966–7	2·9	3·1	119	35

Rumen fluid was withdrawn by gentle suction from the ventral sac of the rumen through a rigid tube placed at a constant depth of 18 in. below the lower edge of the cannula. The first sample was taken immediately before giving the morning feed of concentrate at 6 a.m. The hay was given 15–20 min later and further samples of rumen fluid were taken $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4, 6, 8 and $9\frac{1}{2}$ h after the concentrate feed. Sets of samples were taken at intervals of 1, or more usually 2 weeks, until 2 successive sets of analyses showed little change. This resulted in treatment periods of from 5 to 9 weeks, followed by a gradual change to the next diet during 1 week. Water was available to the animals at all times.

The rumen fluid was first strained through 2 and then 4 layers of butter-muslin to remove suspended material, cooled to 20 °C and the pH value measured. Portions of the fluid were immediately taken for the determination of total volatile fatty acids (VFAs), total N, NPN and ammonia N. Total VFAs were determined by the method of Annison (1954), taking 4 successive 40-ml distillates from each sample. These were titrated with 0·01 N-NaOH, the value for the fourth distillate being considered the 'blank' to be deducted from the other 3 readings. After titration the first 2 distillates were mixed, made alkaline and evaporated to dryness. The residue was moistened with 0·05 ml water and half of this volume was used for the estimation of the individual VFAs by the method of James & Martin (1952). For the total N determination, approximately 10 g rumen liquor were diluted to 50 ml. and the N measured by a semi-micro Kjeldahl method using a mercury catalyst. Protein and any finely suspended material were precipitated by diluting approximately 10 g rumen liquor to 100 ml with a 0·75% solution of sodium tungstate in 0·075 N H_2SO_4. After standing for 1 h the liquid was filtered without suction through no. 42 Whatman paper and the NPN in the filtrate determined by the semi-micro Kjeldahl method. A value for the ammonia N was obtained after making alkaline 20 ml of the NPN filtrate and then steam distilling for 10 min, the distillate being collected in 1% H_3BO_3 and titrated with 0·01 N-HCl. Total solids were weighed as the residue left after evaporating approximately 5 g of the strained rumen fluid to dryness on a steam bath followed by 3 h in an oven at 100 °C.

RESULTS

The solubility at 39 °C of the biuret used in the concentrates, measured approximately in distilled water and in filtered rumen fluid that had been held at 80 °C for 10 min, was 2·1 and 2·3%, respectively; urea at 25 °C has a solubility of 119 g/100 ml.

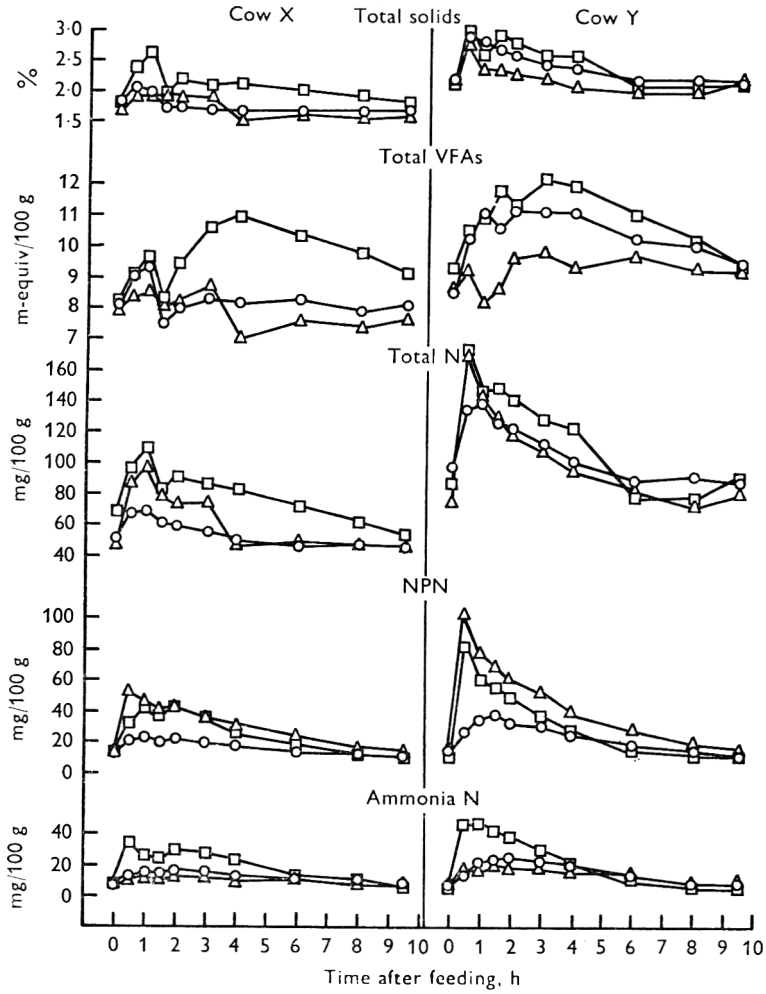

Fig. 1. Changes in the composition of the rumen fluid after feeding (1965–6). ○, Control concentrate; △, biuret concentrate; □, urea concentrate.

The results of the analyses of the rumen liquor for each animal for the last 2 sampling runs on each diet in 1965–6 have been averaged and are shown in Fig. 1. The urea and biuret each supplied 52% of the concentrate N and approximately 36% of the total dietary N. The concentrates were usually consumed in 6–15 min and the hay in 45–90 min, with cow X being a considerably slower eater than cow Y, a characteristic that probably influenced the peak values recorded for this cow in the first 1–2 h of the sampling period.

Total solids. As might be expected, a rise in the solids content of the strained rumen

fluid from both cows occurred immediately after feeding. The effect was greater in the liquor from cow Y and, although this cow was receiving less food than cow X throughout these experiments, the rumen fluid from cow Y consistently had a higher solids content than that of cow X. Such individual variation was presumably associated with differences in rate of passage of the digesta and of water retention in the 2 cows. For both animals, consumption of the urea diet resulted in the highest content of total solids in the rumen fluid and consumption of the biuret diet in the lowest total solids.

Total VFAs. This was the constituent of the rumen fluid that showed considerable variation between successive sampling runs, and usually within a single run, as has been recorded by other workers such as Annison (1954) and Bath & Rook (1963). The double peak form of curve shown in Fig. 1 occurred on all occasions when concentrates were fed but was much less pronounced, or entirely absent, when only hay or dried grass was eaten. This suggests that the first peak may have arisen from the VFAs generated from the starch and sugars of the concentrate and the second, broader peak from the more gradual metabolization of the structural carbohydrates of the hay. The VFA curves for cow X, which ate more slowly than did cow Y, show the double peak more clearly, which would be in agreement with the above suggestion. Additionally, the large volume of water invariably drunk towards the end of the hay eating period, 1–1½ h after the hay was provided, could have caused some dilution of the VFA concentration about that time. The concentration of VFAs in the rumen fluids was slightly greater for cow Y than for cow X, and for both cows the highest values were recorded when the urea diet was given and the lowest values with the biuret diet. VFA concentration was at a generally high level between 2 and 5 h after giving the feed (Fig. 1).

Total N, NPN and ammonia N. Although the total N in rumen fluid is derived from a variety of sources, including the rumen flora, feed residues incompletely broken down, digestion products of the feed and recently ingested food, the curves for total N shown in Fig. 1 have a relatively simple shape governed chiefly by the concentrate intake immediately after the first sample. As would be expected, the more readily available N from the biuret and urea concentrates gave rise to appreciably greater amounts of total N than did the control, oilcake concentrate. The maximum concentration was reached in 1 h for the slower-eating cow X and in 30 min for cow Y, and was much higher for the latter. Even if the total N figures are expressed as per g of total solids, to allow for the higher level of total solids in the rumen liquor from cow Y, the difference in the values obtained for the 2 cows was still large. The urea diet gave rise to the highest amount of total N in the rumen fluid of both cows for most of the sampling period. This could have been because the low solubility of the biuret and its relatively high density (1·4) allowed some sedimentation of biuret to occur. The concentration of total N in the rumen fluid on all 3 diets had fallen to the prefeeding level after about 6 h.

The concentration of NPN was slightly lower from the urea diet than from the biuret diet. This may have been the result of greater absorption of ammonia from the rumen fluid following the urea diet, which produced concentrations of ammonia N almost double those from the biuret diet during the first 2 h after feeding. The control diet gave rise to the lowest concentrations of NPN in the rumen fluids but resulted

in ammonia N concentrations very slightly higher than those from the biuret diet. There was little change in ammonia N concentration on any of the diets after 6–7 h from the time of feeding.

In addition to the actual concentration of NPN and ammonia N in the rumen fluid, the proportions of these substances relative to the total N and to each other throughout the day are also probably of importance. Figure 2 shows these relation-

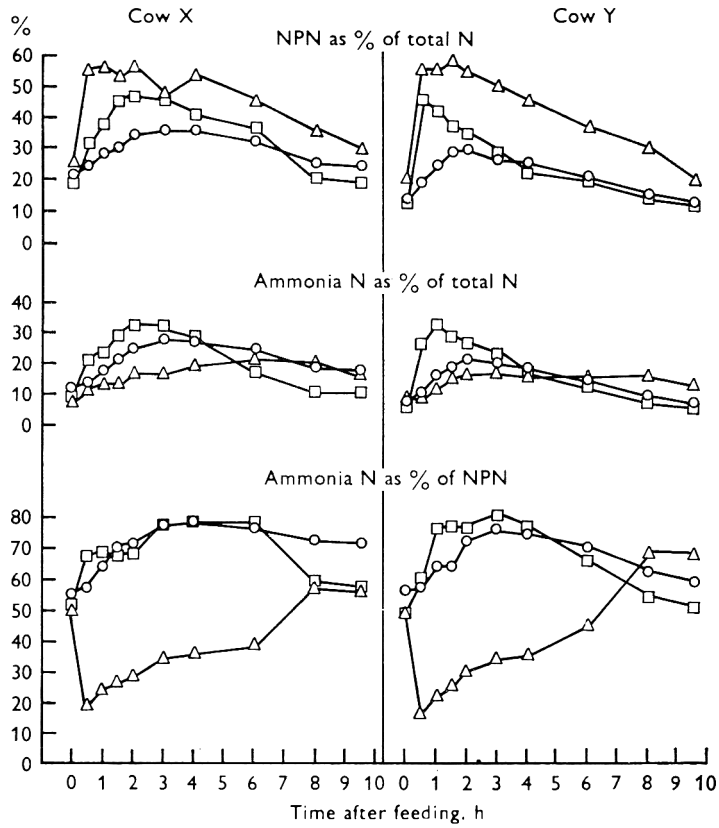

Fig. 2. Changes in the proportions of the N fractions after feeding. ○, Control concentrate; △, biuret concentrate; □, urea concentrate.

ships which emphasize the effect of the low solubility of the biuret, and possibly of its slower rate of conversion to ammonia, in maintaining a high proportion of the total N as NPN, and the low proportion of ammonia N, particularly in the NPN. The urea diet produced a higher proportion of ammonia N in the NPN than did the control diet only during 30–60 min after feeding for cow X and during 1–3 h for cow Y. It was thought that this result might have arisen from the relatively high solubility of the N in groundnut meal used in the control concentrate but in the second year's work, when a mixture of oilcakes with less soluble N was used, the results were only slightly different (Table 2).

The figures for ammonia N as a percentage of the NPN afforded some evidence of adaptation of the rumen flora to biuret. The values for individual sampling runs

made 1, 3, 5 and 8 weeks after cow Y had changed completely to the biuret concentrate, given in Fig. 3, show that an increase occurred in the proportion of ammonia N, presumably as the result of more complete conversion of the biuret. From this evidence and the evidence of similar results for cow X, in both years, it seems that full adaptation to biuret in these animals took 5–8 weeks.

The experiment so far described was repeated without change of technique in 1966–7 using concentrates in which the total N content had been reduced to 87% of that employed previously but without change in energy content (Waite *et al.* 1968). The concentrations of urea and biuret were thus reduced to 2·1 and 2·6% of the

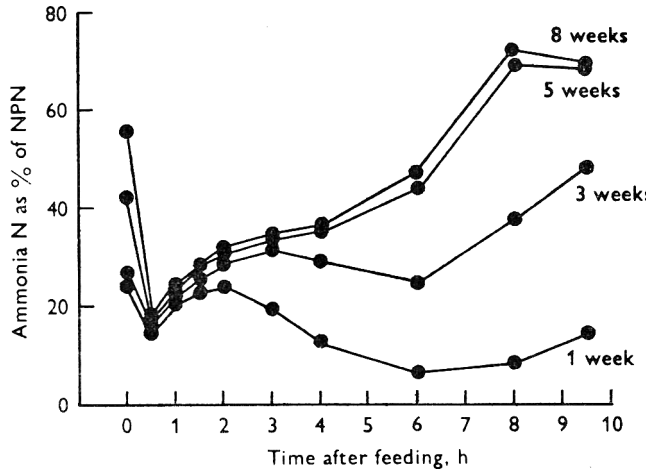

Fig. 3. The increasing proportion of ammonia N in the NPN as biuret feeding progressed.

concentrate, providing 43% of the concentrate N. It is sufficient to record that exactly similar results were obtained, with the fluid from cow Y again containing greater amounts of all the constituents than did that from cow X. The latter cow ate the concentrates more quickly in the second year than it did in the first, and the curves for the concentrations of total solids and the N constituents plotted against time after feeding showed more clearly defined peaks, similar to those for cow Y.

The results of the last 2 sampling runs with each diet have been averaged for each of the 2 years and are presented in Table 2. The mean values of the 2 runs were usually quite close and this reproducibility was independent of the dietary treatments. To avoid biasing the mean value by the 5 results obtained during the first 2 h of the sampling run, the mean was calculated from the curves shown in Fig. 1, taking values at half-hourly intervals. From the values given in Table 2 it can be seen that the use of biuret, compared with the control and urea concentrates, tended to reduce the concentration of total solids and total VFAs, to increase the concentration of NPN and to lower the content of ammonia N. Compared with the urea diet the total N in the rumen fluid on the biuret diet contained an appreciably higher proportion of NPN and a lower proportion of ammonia.

Composition of the total VFAs. The difference in the effects of the 3 concentrates on the proportions of acetic, propionic, butyric and valeric acids in the total VFAs was

small, as can be seen from Table 3 which gives the mean values for the 20 samples taken during the last 2 runs with each concentrate. Valeric acid (iso-+n-) accounted for only 1–3% of the total acids and iso-butyric acid was often absent or present only in traces.

Table 2. *Analysis of the rumen fluid*

Cow	Concentrate	Total solids, %	Total VFA m-equiv/ 100 g fluid	Nitrogen, mg/100 g fluid			NPN as % of total N	Ammonia N as % of total N	Ammonia N as % of NPN
				Total	NPN	Ammonia			
				1965–6					
X	Control	1·87	8·5	62·0	17·8	12·3	28·7	19·8	69·1
	Biuret	1·71	7·5	61·2	30·3	9·3	49·5	15·2	29·7
	Urea	2·10	9·5	79·1	26·3	17·9	33·3	22·6	68·1
Y	Control	2·47	10·4	109·4	24·2	17·4	22·1	15·9	72·0
	Biuret	2·24	9·4	109·1	49·3	15·8	45·1	14·5	32·1
	Urea	2·53	10·9	116·9	33·6	23·5	28·7	20·1	70·0
				1966–7					
X	Control	1·99	8·9	59·9	10·7	6·2	17·7	10·4	58·1
	Biuret	1·84	8·5	59·2	18·0	5·1	30·4	8·6	28·3
	Urea	1·95	9·9	61·6	13·5	9·2	21·7	14·9	68·4
Y	Control	2·19	9·3	76·6	14·4	9·8	18·8	12·8	68·0
	Biuret	2·15	9·2	96·1	24·1	10·7	25·1	11·1	44·5
	Urea	2·32	9·4	85·5	16·4	11·1	21·9	14·9	68·1
Average difference between means of the 2 sampling runs		0·07	0·7	3·4	1·9	1·3	3·5	2·6	2·5

Table 3. *The individual volatile fatty acids in the rumen fluid*

Cow	Concentrate	Total VFA, m-equiv/ 100 g fluid	Acetic, M %	Propionic, M %	Butyric, M %	Valeric, M %	pH
			1965–6				
X	Control	8·5	70·6	15·2	12·9	1·2	6·75
	Biuret	7·5	69·7	15·8	13·5	1·0	6·79
	Urea	9·5	68·9	16·5	14·0	0·6	6·69
Y	Control	10·4	67·3	16·3	15·0	1·5	6·65
	Biuret	9·4	68·6	14·0	16·4	1·0	6·66
	Urea	10·9	67·1	15·9	16·5	0·5	6·65
			1966–7				
X	Control	8·9	68·3	16·4	13·2	2·1	6·81
	Biuret	8·5	67·2	16·8	14·4	1·7	6·85
	Urea	9·9	67·5	17·2	13·0	2·3	6·75
Y	Control	9·3	67·5	15·3	14·6	2·6	6·68
	Biuret	9·2	67·0	15·5	14·8	2·7	6·64
	Urea	9·4	66·0	15·1	16·2	2·7	6·63

The variation in the proportions of the acids after feeding the concentrate diets followed the pattern noted by earlier workers (see, for example, Gray & Pilgrim, 1951) of a decreasing percentage of acetic acid and an increasing percentage of propionic during the first 4 h after feeding, followed by a slower reversal of these trends during the remainder of the sampling period. The percentage of butyric acid rose slightly during the first 1–2 h after feeding, but thereafter changed only slowly

over several hours. Because the 3 diets produced such small differences in the proportions of any given individual fatty acid, variation in the concentration of the individual acids in the rumen liquor at any time was governed mainly by the changes that occurred in the concentration of the total VFAs.

Variation in pH value of the rumen fluids after feeding closely reflected the rise in total VFAs, the value falling by 0·2–0·4 pH units to its lowest levels after 4 h. The differences resulting from the 3 feeding treatments were small (Table 3).

The large protozoa were visible as a sediment in all rumen fluids on standing, the amount of sediment reaching a maximum 90–120 min after giving the concentrate.

DISCUSSION

Biuret was used as a source of nitrogen in the feeding experiments with milking cows (Waite *et al.* 1968) in the expectation that its relatively low solubility in rumen fluid (about 2·3%) would minimize the rapid evolution of ammonia that is characteristic of urea feeding. The results shown in Fig. 1 and the summarized values in Table 2 for both cows in 2 separate years with differing intakes of total and biuret N demonstrate that this expectation was realized. Although the peak concentration of NPN in the rumen fluid following the biuret treatment was the highest obtained on any of the 3 treatments, and usually remained at the highest values throughout the sampling period, the ammonia N concentration was the lowest. If the results for the 2 cows (Table 2) are averaged, then in 1965–6 the control treatment resulted in the appearance of 18% ammonia N in the total N, the biuret treatment in 15% and the urea treatment in 21%. In 1966–7, when biuret and urea formed a smaller proportion of the diets the corresponding values were 11, 10 and 15%, respectively. In the previous paper (Waite *et al.* 1968) it was found that the biuret treatment was not superior to the urea treatment for milk production and milk composition and, assuming that the cows in those trials responded to the diets in a manner similar to that of cows X and Y, a possible explanation may be that the differences between the 2 treatments in the concentration of ammonia N in the rumen fluids were too small. In 1965–6 the biuret treatment produced approximately 8 mg ammonia N/100 g rumen fluid less than did the urea treatment, i.e. 7–12% of the total N in the rumen liquor, and in 1966–7, at the lower level of NPN in the concentrates, the difference was still less, 1–4% of the total N. The peak concentrations, however, are likely to be of more importance than the over-all average and here the differences were greater. With diets containing 2·9% of urea (1965–6) the rumen fluid from cow X at 3 h after feeding contained approximately 18 mg ammonia N/100 g more than it did after giving the biuret diet, and for cow Y the corresponding difference was 24 mg ammonia N. When the level of urea in the concentrate was reduced to 2% (1966–7) the corresponding differences were 16 and 25 mg ammonia N for the 2 animals during the 2-h period following feeding.

The ammonia production resulting from urea feeding can, of course, be minimized by increasing the frequency of feeding the concentrate. When only half the normal quantity of urea concentrate was fed at 6 a.m. and the other half at 10 a.m., the curves with a single peak shown in Fig. 1 for the N constituents were changed to curves showing 2 smaller peaks about 1 h after each feed, and the average NPN and

ammonia N contents over the whole sampling period were each reduced by approximately 30% without change in the mean total N content.

Holzschuh & Wetterau (1965) have shown that even when the ammonia N in rumen fluid of cows given NPN orally rose from 5 to 140 mg/100 g, blood urea concentration did not rise appreciably until 1½ h after the peak rumen ammonia concentration had been attained and then increased only from 9 to 13 mg urea/100 g blood. This suggests that the relatively small differences in peak ammonia N concentration resulting from the present urea and biuret treatments were unlikely to have caused appreciably different blood urea concentrations. If this were so, then wastage of feed N as urinary urea would be similar on the 2 diets and biuret would be no more efficient than urea as a source of N for the cow. Where labour is available, the advantages expected from biuret could probably be achieved by increasing the frequency of feeding a urea concentrate. It is, however, well established (Rowland, 1946; Holmes, Waite, MacLusky & Watson, 1956) that milk production is not particularly responsive to small changes in nitrogen intake when the feed energy supplied is adequate.

The need for a period of adaptation before the rumen flora can utilize NPN compounds to the best advantage of the host animal has been demonstrated by many workers (see, for example, Hatfield, Garrigus, Forbes, Neumann & Gaither, 1959; Schaadt, Johnson & McClure, 1966; Virtanen, 1966). The present results with biuret indicate that during such an adaptation period the amount of ammonia released from biuret increased (Fig. 3). For cow Y in 1965–6, the average concentration of ammonia N in the rumen liquor rose from 7·4 mg/100 g after the animal had received biuret concentrates for only 1 week to 12·0 mg in the 3rd week, 15·0 mg in the 5th week and 16·0 mg in the 8th week. In the milking trials already referred to (Waite *et al.* 1968, expt 2) the fall in milk yield during the continuous feeding experiment decreased after the cows had received biuret concentrates for 5 weeks and the above results offer a possible explanation for that improvement.

There were no significant differences in the total VFA concentrations or in the distribution of the individual acids within the total resulting from the 3 concentrates for either cow, and, as the energy values of the 3 diets were the same and N was not deficient, this was not unexpected. The pattern of change in concentration of the total VFAs during the sampling period was much the same as had been recorded previously (Annison, 1954) changing appreciably in the first 4 h after feeding. We would therefore support the view of Bath & Rook (1963) that analysis of a single sample of rumen fluid drawn at any time throughout the feeding cycle is inadequate to characterize the total VFA concentration produced from the diet and, as can be seen from the results shown in Fig. 1, this is also true of the N fractions. For the 2 h following a feed, particularly one containing concentrates, the content of most compounds in the rumen liquor is likely to be changing rapidly but if, for any reason, a single sample only has to be analysed, one taken about 3 h after feeding would, from the present results, be expected to give reasonably reproducible results from cows adapted to the diet.

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The detection of abnormal milk by electrical means

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Summary. A preliminary study has been made of some relationships between the relative conductivity of milk and its cellular content and 3 basic relationships subjected to an extensive computer analysis. The results suggest that relative conductivity can be used to detect about 3 out of every 4 cows yielding milk samples from one or more quarters with a cellular content greater than 500000/ml, and about 4 out of every 5 cows with a cellular content greater than 1000000/ml. Less than 1 in 20 cows would be incorrectly detected as having cell counts greater than 500000/ml in milk from any one quarter.

Bovine mastitis constitutes the greatest single cause of loss of milk in the dairy industry. The cost to the British industry has been estimated at over £20 million p.a. (Blackburn, 1962). The average incidence of subclinical mastitis, where the milk is not visibly affected, varies from about 8% of the quarters in milk, for a herd in which there have been regular tests and treatment, to about 25% or more in untreated herds (Blackburn, 1959). These statistics and the present trend towards larger dairy herds, higher quality milk, narrowing profit margins for milk production, and automation, indicate a need for a device for the automatic detection of abnormal milk while the cow is being milked. Such a device would only be feasible if a relationship exists between an accepted index of abnormality and some other property of abnormal milk that is amenable to relatively simple measurement.

This paper embodies the results of a preliminary study of the various relationships that exist between the electrical conductivity of milk and its cellular content, and of the feasibility of detecting abnormal milk by electrical methods.

EXPERIMENTAL

During the past 18 months the authors have investigated 17 different physical measurement techniques for the investigation of the properties of abnormal milk. From these studies it was concluded that the only method of potential value for a practical device was the measurement of electrical conductivity. Early workers in this field concentrated on the relationship between absolute electrical conductivity and the chloride content of milk. However, the electrical conductivity of the milk in an udder will be influenced by such factors as diet, breed, temperature and phase of lactation cycle. Davis (1947) suggested that these factors might be eliminated if the conductivity reading of the milk from one quarter were interpreted in relation to the

difference between it and the lowest reading for any other quarter of the same udder. This suggestion was pursued by Jones (1949). With Mr Jones's permission it was decided to use his original data as the basis for the present analysis.

In this paper, the total cellular content/ml is used as the 'true' indicator of abnormality whilst the diagnosis given by the conductivity measurements is termed the 'measured abnormality'. The object is to obtain the optimum agreement between the measured abnormality and the true abnormality. Blackburn, Laing & Malcolm (1955) have reported that the differential cell count possesses little or no advantage over the total cell count in the diagnosis of abnormality. The cell count was made by the Breed method using a standard wire and counting 30 fields at random. Because of the time involved, the counting had to be carried out by more than one person.

The milk samples were categorized according to their cellular content. The cell count categories were: 0–200000, 200000–500000, 500000–1000000, 1000000–5000000 and > 5000000. The criterion of true abnormality can be placed at any of the boundaries of these categories, according to the degree of abnormality being sought. The 5 categories correspond roughly to normal milk and milk from quarters with mild subclinical mastitis, with average subclinical mastitis, with severe subclinical mastitis, and with clinical mastitis, respectively.

Jones (1949), using a single set of criteria, concluded that abnormal milk is not necessarily detected by electrical conductivity. However, with the aid of a digital computer it is now possible in numerous ways to vary the criteria for assessing the measured abnormality.

There are a number of combinations of quarter-sample conductivity data that can be considered for analysis and these are still being studied. For the present analysis 3 different methods were used and their results compared to assess their relative value as indicators of abnormality. The 3 methods were based on the following criteria:

(1) The difference between the specific conductance of a particular quarter sample and that of a sample from the quarter giving the lowest value. (This was the basic criterion adopted by Jones.)

(2) The ratio of the specific conductance of a particular quarter sample to that of the quarter sample having the lowest value. This ratio will be referred to as the 'sample ratio'.

(3) The ratio of the highest to the lowest specific conductances of the milks from the different quarters. This will be referred to as the 'limit ratio'.

ANALYSIS OF RESULTS

Method 1. The difference between the specific conductance of a quarter milk and that of the quarter milk with the lowest specific conductance in the udder is called the 'degrees above normal' (D.A.N.) value. The greater the D.A.N. value, the greater the measured abnormality of the quarter. The lowest specific conductance in the udder may itself be abnormally high, in which case the D.A.N. values of the other 3 quarters would have little significance. To guard against this contingency, the lowest reading should be smaller than a predetermined highest acceptable 'base value'. If the lowest reading is larger than this base value, the D.A.N. values of all 4 quarters are calculated by using the base value as the 'lowest reading'.

The D.A.N. value required to give a measured abnormality was varied from 1·0 to 15·0 inclusive by steps of 1·0 ($\times 10^{-4}$ mho/cm). For each D.A.N. value the highest acceptable base value was varied from 48×10^{-4} to 62×10^{-4} inclusive, by steps of 1×10^{-4} mho/cm. For each combination of D.A.N. value and base value the measured abnormality was compared with the true abnormality as indicated by the total cell count, considered in relation to the various cell-count category boundaries. For each cell-count category, this procedure enables the combination of D.A.N. value and highest acceptable base value to be selected which gives the maximum proportion of correct diagnoses. Table 1 summarizes the results by this method.

Table 1. *Results of method* 1

	True diagnosis		Measured diagnosis					
			Normal		Positive			
Cell category	No. of normal quarters	No. of positive quarters	Correct	Incorrect	Correct	Incorrect	D.A.N. value	Highest acceptable base value
> 200000	181	459	57	115	344	124	1·0	49
> 500000	413	227	393	134	93	20	6·0	53
> 1000000	507	133	494	77	56	13	8·0	55
> 5000000	610	30	590	15	15	20	12·0	60

This table is best explained by describing the first line. The first column contains the figure 200000, which is a total cellular content/ml. For this line of the table, if a quarter sample has a cell count higher than 200000/ml it is regarded as truly abnormal. There were 181 quarters with cell counts of less than 200000 and 459 with cell counts greater than 200000. Thus, 459 quarters would have been diagnosed as abnormal on a total cell-count basis. The best measured diagnosis was obtained by taking a D.A.N. value of 1·0 and a base value of 49×10^{-4} mho/cm. Of those quarters which had a measured diagnosis of normal, 57 were correctly diagnosed and 115 were diagnosed as normal when they were truly abnormal on a cell-count basis. Of those quarters which had a measured diagnosis of abnormal, 344 were correctly diagnosed and 124 incorrectly diagnosed.

The efficacy of method 1 for detecting normal quarters is given by the ratio (correct negative diagnoses)/(total number of negative quarters). The efficacy for detecting positive quarters is given by the ratio (correct positive diagnoses)/(total number of positive quarters). The efficacies are as follows:

For negative quarters

31% for cell counts < 200000
95% for cell counts < 500000
97% for cell counts < 1000000
97% for cell counts < 5000000

For positive quarters

75% for cell counts > 200000
41% for cell counts > 500000
42% for cell counts > 1000000
50% for cell counts > 5000000

For cell counts greater than 5000000, the best combination of D.A.N. value and highest acceptable base value was not so obvious as for the other cell-count category boundaries. As the D.A.N. value is increased, the number of false positives decreases and the number of false negatives increases.

Method 2. The greater the sample ratio, the greater is the measured abnormality of the quarter. However, if the quarter has a specific conductance that is greater than a predetermined upper conductivity limit, the quarter is regarded as having a measured abnormality irrespective of the ratio value.

Table 2. *Results of method* 2

Cell category	True diagnosis		Measured diagnosis				Sample ratio	Upper conductivity limit
	No. of normal quarters	No. of positive quarters	Normal		Positive			
			Correct	Incorrect	Correct	Incorrect		
> 200000	181	459	42	80	379	139	1·00	50
> 500000	413	227	390	132	95	23	1·15	58
> 1000000	507	133	496	84	49	11	1·20	62
> 5000000	610	30	605	22	8	5	1·40	80

The ratio value required to give a measured abnormality was varied from 1·00 to 1·80 by steps of 0·05. For each ratio value the upper conductivity limit was varied from 82×10^{-4} to 50×10^{-4} by steps of 2×10^{-4} mho/cm. For each combination of ratio value and upper conductivity limit, the measured abnormality was compared with the true abnormality as assessed from the cell count category boundaries. For each boundary the combination of ratio value and upper conductivity limit that gave the maximum proportion of correct diagnoses was selected. Table 2 summarizes the results.

There is no significant difference between the results by method 2 and those by method 1. This conclusion is still valid even if no upper conductivity limit is imposed in method 2. For method 2 itself, the imposition of an upper conductivity limit gives significantly improved results for cell counts > 200000 and > 500000. There is no significant improvement with the other 2 category boundaries.

Figure 1 illustrates the effect on the number of false diagnoses when different upper conductivity limits are imposed. The sample ratio has been held constant at 1·15 and cell counts > 500000 are truly positive. Figure 2 illustrates the effect on the number of false diagnoses when the sample ratio is varied. The upper conductivity limit has been held constant at 58·0 and cell counts > 500000 are truly positive. Figure 3 shows, for increasing sample ratio, the distribution of correct and false diagnoses in the case where cell counts > 500000 are truly positive. Figure 4 shows the same distribution in the case where cell counts > 1000000 are truly positive.

The efficacy of method 2 for detecting negative quarters and positive quarters is as follows:

For negative quarters

23% for cell counts < 200000
95% for cell counts < 500000
98% for cell counts < 1000000
99% for cell counts < 5000000

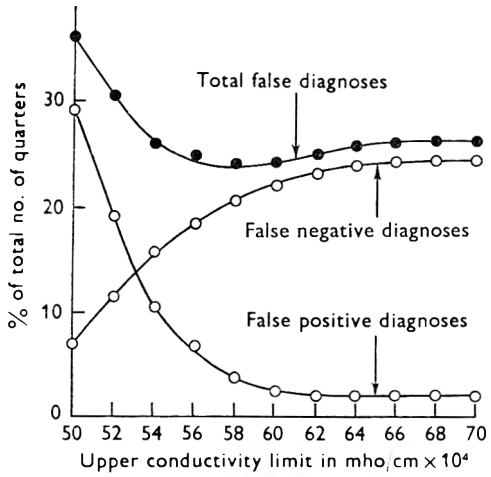

Fig. 1

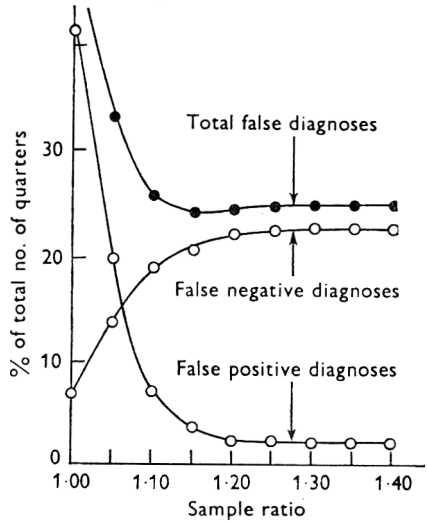

Fig. 2

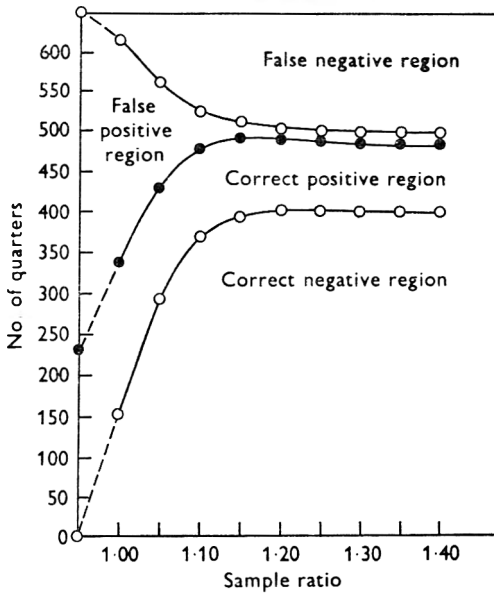

Fig. 3

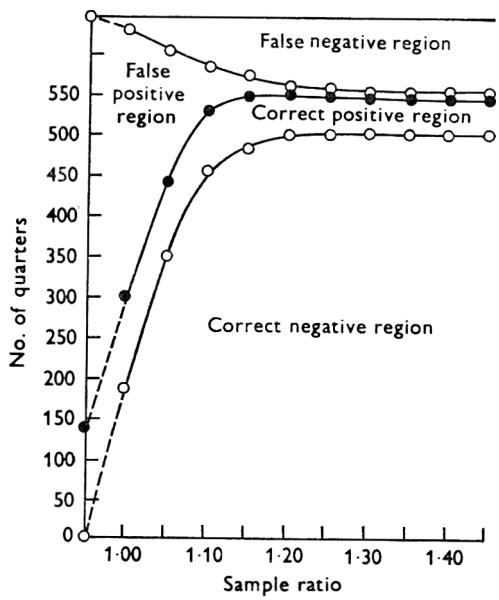

Fig. 4

Fig. 1. Method 2 with the *sample ratio* held constant at 1·15. Distribution of false diagnoses with increasing upper conductivity limit. Cell counts > 500000 are truly positive. Cell counts ⩽ 500000 are truly negative. Minimum number of false diagnoses occurs at an observed upper conductivity limit of 58·0.

Fig. 2. Method 2 with the *upper conductivity limit* held constant at 58·0. Distribution of false diagnoses with increasing sample ratio. Cell counts > 500000 are truly positive. Cell counts ⩽ 500000 are truly negative. Minimum number of false diagnoses occurs at a sample ratio of 1·15.

Fig. 3. Method 2 with the upper conductivity limit held constant at 58·0. Distribution of correct and false diagnoses with increasing sample ratio. Cell counts > 500000 are truly positive. The maximum number of correct diagnoses occurs at a sample ratio of 1·15.

Fig. 4. Method 2 with the upper conductivity limit held constant at 62·0. Distribution of correct and false diagnoses with increasing sample ratio. Cell counts > 1000000 are truly positive. The maximum number of correct diagnoses occurs at an observed sample ratio of 1·20.

For positive quarters

83% for cell counts > 200000
42% for cell counts > 500000
37% for cell counts > 1000000
27% for cell counts > 5000000

Method 3. The efficacies of methods 1 and 2 for detecting positive quarters are encouraging but somewhat low. It was considered that an improvement might be made by working on a whole cow basis rather than on a quarter basis. In this case the limit ratio is used and selection is for positive cows instead of positive quarters.

The greater the limit ratio, the greater the measured abnormality of the cow. However, if the highest specific conductance in the udder is greater than the upper conductivity limit, the cow is regarded as having a measured abnormality irrespective of the limit ratio value. For each cell count category the combination of ratio value and upper conductivity limit that gave the maximum proportion of correct diagnoses was selected. Table 3 summarizes the results.

Table 3. *Results of method* 3

Cell category	True diagnosis		Measured diagnosis				Limit ratio value	Upper conductivity limit
	No. of normal cows	No. of positive cows	Normal		Positive			
			Correct	Incorrect	Correct	Incorrect		
> 200000	12	148	0	0	148	12	1·00	54
> 500000	65	95	60	40	55	5	1·15	60
> 1000000	93	67	83	21	46	10	1·15	62
> 5000000	141	19	133	8	11	8	1·30	70

In this case the imposition of an upper conductivity limit gives no significant improvement in the results. This conclusion is important because it implies that the instrumentation in the proposed device is considerably simplified.

The efficacies of method 3 for detecting negative and positive cows are as follows:

For negative cows

0% for cell counts < 200000
92% for cell counts < 500000
89% for cell counts < 1000000
94% for cell counts < 5000000

For positive cows

100% for cell counts > 200000
58% for cell counts > 500000
69% for cell counts > 1000000
58% for cell counts > 5000000

The over-all efficacy of the method at the cell count boundary of 200000 is zero since it has detected all the positive cows by diagnosing all the cows as positive. If the first cell-count category is omitted and no upper conductivity limit is imposed, method 3 yields the results given in Table 4. The table also shows that nearly all the false positive diagnoses are developing cases, and a very high proportion of the false

negative diagnoses are only positive on a cell-count basis by less than one category. This clearly has an important bearing on the calculated efficacy of method 3.

Figures 5–7 show, for increasing limit ratio and no upper conductivity limit, the distribution of correct and false diagnoses for the different cell-count category boundaries.

Table 4. *Simplified version of method* 3

	True diagnosis		Measured diagnosis						
			Normal			Positive			
Cell category	No. of normal cows	No. of positive cows	Correct	False	No. of false negatives in lowest positive category	Correct	False	No. of false positives in highest negative category	Limit ratio value
> 500000	65	95	61	45	22	50	4	4	1·15
> 1000000	93	67	83	23	21	44	10	6	1·15
> 5000000	141	19	135	9	9	10	6	5	1·30

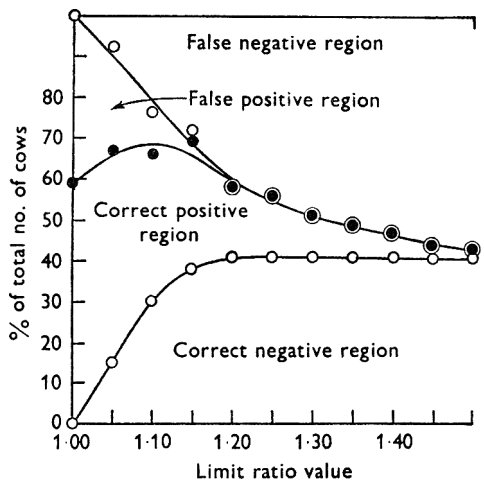

Fig. 5

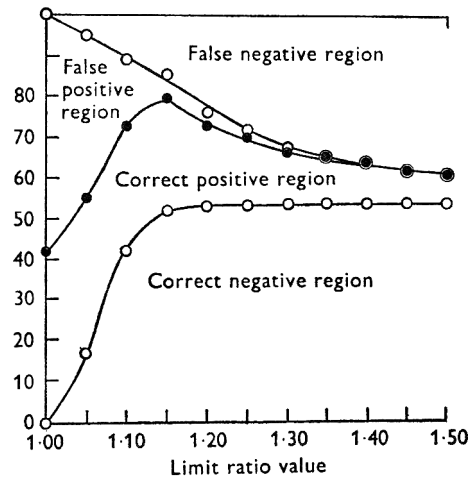

Fig. 6

Fig. 5. Method 3 with no upper conductivity limit. Distribution of correct and false diagnoses with increasing limit ratio. Cell counts > 500000 are truly positive. Cell counts ⩽ 500000 are truly negative. The maximum number of correct diagnoses occurs at an observed limit ratio of 1·15 and at an estimated ratio of about 1·12.

Fig. 6. Method 3 with no upper conductivity limit. Distribution of correct and false diagnoses with increasing limit ratio. Cell counts > 1000000 are truly positive. Cell counts ⩽ 1000000 are truly negative. The maximum number of correct diagnoses occurs at an observed limit ratio value of 1·15.

DISCUSSION

It is important to realize that the figures quoted in Tables 1–4 give an over-pessimistic picture of the effectiveness of conductivity measurements in the diagnosis of abnormality. In practice, the user of the conductivity apparatus would be aware of the past history of the cow in question and this history must be taken into account as well as the conductivity measurements. For example, a 'normal' cow may have a

high cell count because it is in late lactation or recovering from mastitis. Furthermore, there will be daily variations in both the cell count and the specific conductance. A correct interpretation can only really be made when these variations are taken into account.

Although the direct microscopic method of determining the cellular content has long been accepted as a useful indicator of abnormality, it tends to give inconsistent results. Stryndaka & Thornton (1937) found that the accuracy was such that replicate samples would vary by up to $\pm 50\%$ from the mean, and the highest count was occasionally more than 3 times the lowest count. Thus, it is not realistic to give the cell count categories sharp boundaries. Each boundary could possibly be replaced by a relatively narrow 'doubtful' region.

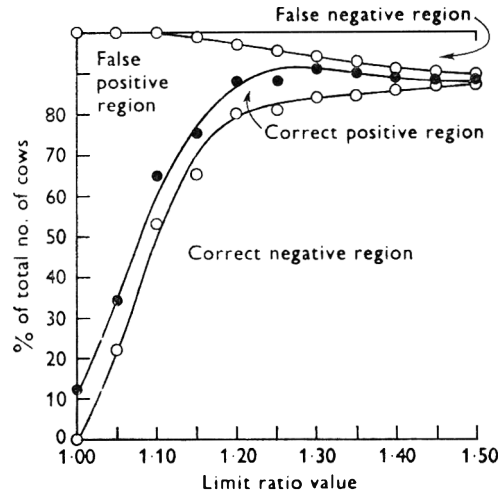

Fig. 7. Method 3 with no upper conductivity limit. Distribution of correct and false diagnoses with increasing limit ratio. Cell counts > 5000000 are truly positive. Cell counts ≤ 5000000 are truly negative. Maximum number of correct diagnoses occurs at a limit ratio of about 1·30.

The efficacy of method 3 for detecting positive cows with cell counts of > 5000000/ml is lower than might be expected. This is due to the failure of conductivity methods with milk from quarters with obvious clinical mastitis. Simple visual inspection of the udder would suffice in these cases.

CONCLUSION

The greatest potential value of a conductimetric test would be in the detection of milks with cell counts in the range 500000–5000000/ml. This is the range of cell counts found in quarters affected with average and severe subclinical mastitis.

If all the 'just positive' cases and all the 'developing positive' cases are neglected, Table 4 will provide a rough lower limit for the efficacy of method 3. If these cases are all credited with a correct measured diagnosis, Table 4 will provide a rough upper limit for the efficacy of method 3. Thus, for a cell-count boundary of 500000/ml the efficacy for detecting positive cows lies between 53 and 76%. The efficacy for detecting negative cows lies between 94 and 100%. For a cell-count boundary of 1000000/

ml the efficacy for detecting positive cows lies between 66 and 97%. The efficacy for detecting negative cows lies between 90 and 96%.

At the present time, cases of subclinical mastitis are rarely detected by the cowman. The results of method 3 suggest that it might be possible to engineer a device that would detect such cases with a fairly high efficacy. Extensive field trial of any such device would be needed before it could be recommended for practical use.

We are indebted to Mr G. Elis Jones who not only provided the original data but also gave much valuable advice that considerably improved the technical presentation of this paper. We thank the Middlesbrough Education Authority for providing facilities for carrying out this work; Mr J. F. Harbourne, The Veterinary Investigation Officer, Ministry of Agriculture, Fisheries and Food, Thirsk, Yorkshire, for much help and advice, and the Mathematics Department of this College for help with the I.B.M. 1620 Mk. II digital computer and for guidance on programming technique. We acknowledge also the encouragement and extensive information given to us by the Hannah Dairy Research Institute, Ayr, Scotland and by the National Institute for Research in Dairying, Shinfield, Reading.

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The effects of dietary tallow and cottonseed oil on milk fat secretion in the cow

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SUMMARY. The effects of the isocaloric replacement of part of the dietary concentrate mixture by either tallow or cottonseed oil on the yield and composition of the milk fat was investigated in 2 feeding experiments with a total of 26 cows in mid-lactation. The concentrates were given with high- or low-roughage diets that supplied either 9·1–9·5 or 1·8–3·2 kg hay/day.

In expt 1, the addition of cottonseed oil to the high-roughage diet increased the yield of milk fat during the first 8 days but decreased it during the last 4 days of a 28-day feeding period. In contrast, the inclusion of tallow in the high-roughage diet resulted in an increased yield of milk fat that was sustained throughout the period of 28 days. In expt 2, the intake of dietary fat was less than it was in expt 1 and the inclusion of tallow or cottonseed oil in either the high- or low-roughage diets had little effect on the yield of milk fat.

The inclusion of either fat in the diet increased the yields and percentages of stearic and oleic acids and, in general, decreased the yields and percentages of the medium-chain fatty acids (10:0, 12:0 and 14:0) in the milk fat. The addition of tallow to the diet did not appear to alter the yields of the short-chain fatty acids (C4–C8, 4:0, 6:0 and 8:0) in the milk fat. When the low-roughage diets resulted in a decreased yield of milk fat, the secretion of all the fatty acids in the milk fat was reduced, but the reduction in the secretion of oleic acid was less than the reductions in the secretions of the other constituent fatty acids.

Increases in the yield of milk fat have been reported when the diet of the cow was supplemented with fats or oils poor in polyunsaturated acids, e.g. tallow, butter, palm oil or coconut oil (McCandlish & Weaver, 1922; Garner & Sanders, 1938; Allen & Fitch, 1941; Peters, Harris, Mulay & Pinkerton, 1961; Brown, Stull & Stott, 1962). Increased yields of milk fat have also been observed when the diet of the cow was supplemented for short periods with vegetable oils rich in polyunsaturated fatty acids (Nevens, Alleman & Peck, 1926; Allen, 1934; Garner & Sanders, 1938; Davis & Harland, 1946). On the other hand, many investigators have observed a decrease in the yield of milk fat when vegetable oils rich in polyunsaturated fatty acids were

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incorporated in the diet of cows over long periods (Garner & Sanders, 1938; Allen & Fitch, 1941). This decrease in the yield of milk fat was also observed when highly unsaturated oils of marine origin, especially cod-liver oil, were incorporated in the diet of the cow (Channon, Drummond & Golding, 1924; Petersen, 1932; Garner & Sanders, 1938; Shaw & Ensor, 1959). The method of incorporating the fats or oils in the diet may determine the effect on milk fat production. For example, Williams, Cannon & Espe (1939) and Williams (1941) observed that more milk fat was produced by cows given whole oil seeds than by cows given a mixture of the extracted seeds and the oil that had been obtained from the seeds. Moore, Hoffman & Berry (1945) demonstrated that the yield of milk fat was reduced when cod-liver oil was given once each day but was unaltered when the same total amount of cod-liver oil was given in the form of several smaller doses during the day. Loosli, Maynard & Lucas (1944) found that the output of milk fat depended on the levels of fat, starch and roughage in the diet. The addition of fat to the diet resulted in the greatest increase in the yield of milk fat when the diet had either a high-roughage or a low-starch content. These findings were not confirmed by Brown *et al.* (1962). However, interpretation of the results of the experiments of Brown *et al.* (1962) is complicated by the fact that the cows on the low-roughage treatment received almost 3 times as much supplementary fat in the diet as did the animals on the high-roughage treatments. To investigate this problem in greater detail, 2 experiments were carried out in which cows were given diets that supplied the same amounts of 2 different types of fat at 2 levels of roughage intake. The effects of these dietary treatments on the yields and compositions of the milk fat are now reported.

EXPERIMENTAL

Experimental animals

Eighteen pedigree Friesian cows were used in expt 1; these animals had calved on average 128 days before the beginning of the experiment. Eight cross-bred cows were used in expt 2; these animals had calved on average 41 days before the beginning of the experiment.

In expt 1, the cows were housed loose and were bedded on wood shavings, whereas in expt 2 the cows were housed in a building equipped with slats and cubicles. The cows were milked twice daily in a parlour, at intervals of 15 and 9 h, and immediately after milking were yoked to allow individual feeding. Their daily rations were given in 2 equal portions. Water was given *ad lib.*

Experimental diets

Expt 1. In this experiment, 9 of the cows were given the high-roughage diets that supplied 9·1 kg/day of mature ryegrass hay in the long form. The other 9 cows were given the low-roughage diets that supplied 1·8 kg/day of the same hay. The composition of the hay is given in Table 1. Three different concentrate mixtures were given to the cows on each of the 2 roughage treatments. The concentrate mixtures that were given to the animals on the high-roughage treatments differed in composition from those that were given to the animals on the low-roughage treatments. On the high-roughage treatments, the concentrate mixtures contained barley, bran

and decorticated, extracted groundnut meal which resulted in a diet of high-fibre and low-starch content. On the low-roughage treatments, the concentrate mixtures contained barley, flaked maize and decorticated extracted groundnut meal which resulted in diets of low-fibre and high-starch content. The high-fat diets were constituted by isocalorically replacing part of the cereals in both concentrate mixtures with either tallow or cottonseed oil, the assumption being made that the digestibility coefficients of the added fats were both 95% (Evans, 1960). The dietary fats

Table 1. *Compositions (as percentages of the dry matter) of the hays used in expts* 1 *and* 2

Expt	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen-free extractives
1	9·1	37·3	2·1	8·0	43·5
2	10·7	31·8	2·1	5·1	50·3

Table 2. *Weight percentage of the major fatty acids in the tallow and in the cottonseed oil used in expts* 1 *and* 2

Fatty acid	Tallow	Cottonseed oil
16:0	30·3	21·2
18:0	27·5	1·8
18:1	32·2	25·2
18:2	0·4	50·0
18:3	—	1·0

Table 3. *Mean daily intakes (kg) of hay and of each of the constituents of the concentrate mixtures given to the cows in expt* 1

	High-roughage treatments			Low-roughage treatments		
	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil
Hay	9·1	9·1	9·1	1·8	1·8	1·8
Barley	4·0	2·1	2·1	4·0	2·5	2·5
Flaked maize	—	—	—	3·4	3·3	3·3
Decorticated extracted groundnut meal	1·4	2·1	2·1	1·6	1·6	1·6
Bran	0·7	0·4	0·4	—	—	—
Molasses	0·4	0·2	0·2	0·2	0·2	0·2
Tallow	—	0·57	—	—	0·52	—
Cottonseed oil	—	—	0·57	—	—	0·52
Mineral mixture	0·34	0·34	0·34	0·34	0·34	0·34

were incorporated at the level of 10% of the concentrate mixture. The fatty acid compositions of the tallow and cottonseed oil are given in Table 2, in which, as elsewhere in this paper, the shorthand designation of Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) is used to denote individual fatty acids. The mean daily intakes of the various dietary components for the 6 feeding treatments are given in Table 3, from which it can be seen that the intakes of fat were similar for the high- and low-roughage treatments. The amounts of concentrates given to the cows were such that the high- and low-roughage diets both supplied the same amount of energy, as cal-

culated from the values given by Evans (1960). The daily intake of energy was maintained at a constant level for each cow throughout the experiment so that the pattern of rumen fermentation should not be altered by variation in the ratio of hay to concentrates.

Expt 2. In this experiment, 4 of the cows were given the high-roughage diets that supplied 9·5 kg/day of mature ryegrass hay in the long form. The other 4 cows were given the low-roughage diets that supplied 3·2 kg/day of the same hay. The composition of this hay is also given in Table 1. Again, 3 different concentrate mixtures were given to the cows on each of the 2 roughage treatments. As may be seen from Table 4, the concentrate mixtures given to the cows on each of the 6 dietary treatments in expt 2 were similar in composition to the concentrate mixtures given to the cows on the corresponding treatments in expt 1. However, in expt 2, the dietary fats were incorporated at the level of 6 % of the concentrate mixture. As in expt 1, the amounts of concentrates given to the cows were such that the high- and low-roughage diets supplied the same amount of energy, and the daily intake of energy was maintained at a constant level for each cow throughout the experiment. The fatty acid compositions of the supplementary fats were the same as in expt 1 (Table 2).

Table 4. *Mean daily intakes (kg) of hay and of each of the constituents of the concentrate mixtures given to the cows in expt* 2

	High-roughage treatments			Low-roughage treatments		
	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil
Hay	9·5	9·5	9·5	3·2	3·2	3·2
Barley	5·4	4·0	4·0	4·2	3·6	3·6
Flaked maize	—	—	—	4·2	3·6	3·6
Decorticated extracted groundnut meal	1·9	1·9	1·9	1·8	1·8	1·8
Bran	0·9	0·9	0·9	—	—	—
Tallow	—	0·45	—	—	0·45	—
Cottonseed oil	—	—	0·45	—	—	0·45
Mineral mixture	0·34	0·34	0·34	0·34	0·34	0·34

Experimental design

The 18 cows used in expt 1 were divided into 2 groups of 9, by pairing them according to milk yield and the fat content of the milk produced in the week before the beginning of the experiment. One member of each pair was then randomly assigned to either the high- or the low-roughage treatments. Within each roughage treatment, each cow was randomly assigned to one treatment sequence of a 3×3 Latin square. Two of the 3 squares had the same treatment sequences and were balanced with the third square for carry-over effects. The duration of each experimental period was 28 days and the change-over from one treatment to another was made abruptly.

In expt 2, the cows were divided into 2 groups of 4 by pairing them according to the yield of milk and the fat content of the milk produced in the week before the beginning of the experiment. One member of each pair was then randomly assigned

to either the high- or low-roughage treatment sequences in a 4×4 Latin square that was balanced for carry-over effects. Since there were only 3 concentrate treatments for the 4 feeding periods, the concentrate mixture containing tallow was given for 2 periods to the cows on the high-roughage treatments and the concentrate mixture containing cottonseed oil was given for 2 periods to the cows on the low-roughage treatments. The duration of each experimental period was 21 days and the change-over between treatments was abrupt.

Sampling and methods of analysis

In both experiments, milk yields were recorded at each milking and the fat contents of the milks were determined by the Gerber method (British Standards Institution, 1955) on 4-day composite samples obtained throughout each experimental period. In expt 1, the contents of total solids in the milk samples were determined gravimetrically (British Standards Institution, 1951). In both experiments, the Reichert, Polenske and iodine values of the milk fat were determined (British Standards Institution, 1958) on the composite samples obtained during the last 4 days of each experimental period.

In expt 2, the concentrations in the milk fat of the fatty acids with 10–18 carbon atoms were determined by gas–liquid chromatography. The fat was extracted (Storry & Millard, 1965) from the composite samples of milk obtained from the last 4 days of each experimental period. Methyl esters of the fatty acids were prepared by the transesterification procedure of Stoffel, Chu & Ahrens (1959), and the relative proportion of each fatty acid was determined in an Argon Gas Chromatograph (W. G. Pye Ltd., Cambridge, England) as described by Moore & Williams (1963). The concentrations in the milk fat of the fatty acids with 4–8 carbon atoms were calculated arbitrarily from the Reichert and Polenske values.

Statistical treatment of results

The results were analysed statistically by the procedure outlined by Cochran & Cox (1957).

RESULTS

All the rations were consumed within 1 h of feeding. Since certain of the treatment effects were not evident in the early part of the experimental periods, the results of expts 1 and 2 will be discussed for the most part with respect to the differences between treatments that were observed during the last 4 days of each period. However, some results for the first 8 days of each period are also included to illustrate the short-term effects of the dietary treatments.

Expt 1. The mean values for the yield and composition of the milk produced by the cows during the first 8 and the last 4 days of the experimental periods are given in Table 5. When the cows were given the high-roughage diets, the inclusion of tallow or cottonseed oil in the concentrate mixture did not affect the yield of milk or the yield of solids-not-fat (S.N.F.), but significantly decreased ($P < 0{\cdot}01$) the percentage of S.N.F. in the milk. When the cows were given the low-roughage diets, the addition of tallow or cottonseed oil to the concentrate mixture resulted in small but significant ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively) increases in the milk yield

Table 5. *Mean daily yields produced during the first* 8 *and the last* 4 *days of each period in expt* 1

		High-roughage treatments				Low-roughage treatments			
	Days of experimental period	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.
Milk yield, kg	1–8	14·3	14·4	14·4	± 0·24	14·9	15·0	14·7	± 0·32
	25–28	13·7	14·0	13·7	± 0·28	13·2	14·0**	13·7*	± 0·22
Milk fat yield, g	1–8	489	560**	521*	± 15·2	455	479	467	± 22·3
	25–28	489	530*	468	± 19·4	449	454	392*	± 21·9
Milk fat content, %	1–8	3·42	3·89	3·62	± 0·088	3·05	3·19	3·18	± 0·108
	25–28	3·57	3·79	3·42	± 0·068	3·40	3·24	2·86	± 0·137
SNF yield, kg	1–8	1·21	1·20	1·20	± 0·018	1·26	1·25	1·20	± 0·018
	25–28	1·16	1·15	1·12	± 0·028	1·12	1·16	1·13	± 0·029
SNF content, %	1–8	8·46	8·33	8·33	± 0·130	8·46	8·33	8·16	± 0·200
	25–28	8·47	8·21**	8·18**	± 0·06	8·49	8·29**	8·25**	± 0·059
Reichert value	25–28	28·9	27·6***	26·7***	± 0·518	30·4	26·7***	23·8***	± 1·06
Polenske value	25–28	3·34	2·38***	2·24***	± 0·403	4·61	2·58***	1·84***	± 0·43
Iodine value	25–28	29·6	33·5***	39·5***	± 0·745	30·4	35·1***	41·4***	± 1·07

*, **, ***, Significantly different ($P < 0·05$, $P < 0·01$, $P < 0·001$, respectively), from the values obtained with the unsupplemented ration.

during the last 4 days of the experimental periods. Again, the percentage of S.N.F. in the milk was significantly reduced ($P < 0{\cdot}01$) when the concentrate mixture given to the cows on the low-roughage treatment contained supplementary fats. When tallow was incorporated in the concentrate mixture given to the cows on the high-roughage treatment, there was an increase ($P < 0{\cdot}01$) in the yield of milk fat during the first 8 days of the experimental period. During the same period a less marked increase ($P < 0{\cdot}05$) in the yield of milk fat was observed when the high-roughage diet was supplemented with cottonseed oil. The effect of supplementary tallow on the yield of milk fat became less marked as the experimental period progressed but even during the last 4 days, the cows given the high-roughage diet containing tallow still produced significantly more ($P < 0{\cdot}05$) milk fat than when they were given the high-roughage, low-fat diet. The effect of supplementary cottonseed oil on the yield of milk fat noted during the first 8 days was not maintained during the entire experimental period. With the cows on the low-roughage treatment, the addition of tallow to the concentrate mixture did not significantly affect the yield of milk fat. When the low-roughage diet was supplemented with cottonseed oil the yield of milk fat hardly changed during the first 8 days, but during the last 4 days of the experimental period, the cows given the low-roughage diet supplemented with cottonseed oil produced significantly less ($P < 0{\cdot}05$) milk fat than they did during the corresponding 4 days on the low-roughage, low-fat diet. The inclusion of tallow or cottonseed oil in the concentrate mixture given to the cows on either the high- or low-roughage treatments resulted in significant decreases ($P < 0{\cdot}001$) in the Reichert and Polenske values and a significant increase ($P < 0{\cdot}001$) in the iodine value of the milk fat. The effects of dietary fat on these values were more pronounced in the milk fat produced by the cows on the low-roughage treatment. Irrespective of the level of roughage intake, the effect of dietary cottonseed oil on these values was greater than that of dietary tallow.

Expt 2. The mean values for the yields of milk and milk fat and the percentage of fat in the milk produced by the cows during the first 8 and the last 4 days of the experimental periods are given in Tables 6 and 7. In these 2 tables (and in Tables 9 and 10), 2 separate series of standard errors are given for comparing treatment means since the concentrate mixture containing tallow was given for 2 experimental periods to the cows on the high-roughage treatment and the concentrate mixture containing cottonseed oil was given for 2 periods to the cows on the low-roughage treatment. The inclusion of tallow or cottonseed oil in the concentrate mixture given to the cows on either the low- or high-roughage treatments had no significant effects on the yield of milk or the yield of milk fat. However, when the cows were given the high-roughage diet supplemented with cottonseed oil, the milk-fat percentage was significantly lower ($P < 0{\cdot}05$) than that observed when the cows were given the low-fat high-roughage diet.

It is of interest to examine the results obtained for the individual cows that were given the low-roughage diets during the experiment. Before the experiment began, these cows were given an ordinary high-roughage diet containing hay and grass silage. Table 8 shows the percentages of fat in the milk produced by these cows when they were given the ordinary farm ration followed by the 3 experimental diets of low-roughage content. With cow no. 1, the substitution of the unsupplemented

low-roughage diet for the farm ration had no effect on the percentage of fat in the milk but the inclusion of tallow or cottonseed oil in the concentrate mixture reduced the milk-fat percentage. With cows nos. 2 and 3, the substitution of the unsupplemented, low-roughage diet for the farm ration markedly reduced the percentage of fat in the milk but the addition of tallow or cottonseed oil to the low-roughage diet

Table 6. *Mean daily yields produced on the high-roughage treatments during the first 8 and the last 4 days of each period in expt 2*

	Days of experimental period	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M. † (1)	S.E.M. † (2)
Milk yield, kg	2–9	20·3	20·1	19·7	±1·10	±0·95
	18–21	19·1	19·7	19·6	±0·77	±0·67
Milk fat yield, g	2–9	717	740	708	±48·5	±42·2
	18–21	702	732	646	±35·9	±31·1
Milk fat content, %	2–9	3·53	3·68	3·58	±0·21	±0·19
	18–21	3·63	3·72	3·30*	±0·12	±0·11

* Significantly different ($P < 0·05$) from the value obtained with the unsupplemented ration.

† S.E.M.: (1) standard errors for comparing the means obtained with the unsupplemented ration and the ration supplemented with cottonseed oil; (2) standard errors for comparing the means obtained for the ration containing tallow with those obtained for the unsupplemented ration or the ration containing cottonseed oil.

Table 7. *Mean daily yields produced on the low-roughage treatments during the first 8 and the last 4 days of each period in expt 2*

	Days of experimental period	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M. † (1)	S.E.M. † (2)
Milk yield, kg	2–9	18·5	18·4	18·6	±1·00	±0·87
	18–21	18·0	17·9	17·3	±0·78	±0·67
Milk fat yield, g	2–9	498	508	456	±60·8	±52·7
	18–21	380	417	366	±63·5	±54·9
Milk fat content, %	2–9	2·68	2·77	2·45	±0·363	±0·313
	18–21	2·18	2·31	2·14	±0·321	±0·278

† S.E.M.: (1) standard errors for comparing the means obtained with the unsupplemented ration and the ration supplemented with tallow; (2) standard errors for comparing the means obtained for the ration containing cottonseed oil with those obtained for the unsupplemented ration or the ration containing tallow.

Table 8. *Fat content (%) of the milk produced by the individual cows in expt 2 when they were given the pre-experimental ration (high-roughage content) followed by the experimental rations of low-roughage content*

	Cow			
Dietary treatment	1	2	3	4
Pre-experimental ration, high roughage	3·82	3·27	3·68	3·87
Experimental rations, low roughage				
Unsupplemented ration	3·90	1·30	1·60	1·95
Ration supplemented with tallow	3·38	2·04	1·90	1·93
Ration supplemented with cottonseed oil	3·10	1·60	1·91	2·11

	High-roughage treatments					Low-roughage treatments				
	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.† (1)	S.E.M.† (2)	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.† (1)	S.E.M.† 2
Fatty acids										
4:0– 8:0	8·1	7·7	7·9	±0·34	±0·29	5·7	5·5	4·2*	±0·50	±0·44
10:0	2·2	1·3	0·6	±1·53	±1·32	1·3	1·0	0·5	±0·80	±0·70
12:0	2·5	1·9	1·4	±0·78	±0·68	2·3	0·9	1·4	±0·78	±0·66
14:0	12·5	8·6*	7·8*	±1·14	±0·99	10·3	6·2**	7·7**	±0·60	±0·52
16:0	37·5	31·8*	33·5*	±1·39	±1·21	35·2	32·9	30·4*	±1·88	±1·63
16:1	3·1	2·7	2·2	±0·36	±0·31	3·6	3·2	2·9	±0·70	±0·61
18:0	8·1	12·6*	12·8*	±1·13	±0·98	6·0	9·3	8·3	±2·55	±2·20
18:1	17·8	26·2*	26·7*	±2·21	±1·91	24·8	32·3	36·4*	±3·92	±3·34
18:2+18:3	2·3	1·6	2·9	±0·25	±0·21	3·9	2·9	3·6	±0·51	±0·44

*, ** Significantly different ($P < 0·05$, $P < 0·01$, respectively,) from the values obtained with the unsupplemented ration.
† See Tables 6 and 7 for explanation of S.E.M. (1) and S.E.M. (2).

Table 10. *Daily yields (g) of the major fatty acids in the milk fat produced during the last 4 days of each period in expt 2*

	High-roughage treatments					Low-roughage treatments				
	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.† (1)	S.E.M.† (2)	Unsupplemented ration	Ration containing tallow	Ration containing cottonseed oil	S.E.M.† (1)	S.E.M.† (2)
Fatty acids										
4:0– 8:0	53·4	53·0	47·8**	±1·18	±1·04	20·3	21·5	14·7	±5·73	±4·95
10:0	14·4	8·6	3·8*	±3·26	±2·85	4·6	4·0	1·8	±4·08	±3·54
12:0	16·8	12·9*	8·6**	±1·49	±1·26	8·3	3·7	4·8	±4·02	±3·48
14:0	82·7	59·1*	47·6*	±8·30	±7·14	37·0	24·4	26·8	±13·5	±11·7
16:0	248·0	219·0*	203·0*	±9·98	±8·66	126·0	129·0	105·0	±26·8	±23·2
16:1	20·6	18·9	13·5	±4·68	±4·09	12·9	12·6	10·1	±5·01	±4·33
18:0	53·1	87·0**	77·8*	±6·45	±5·59	21·7	36·6	28·6	±11·1	±9·63
18:1	117·0	180·0*	162·0*	±18·8	±16·3	88·9	127·0	126·0	±21·2	±18·4
18:2+18:3	14·8	11·2*	17·6*	±1·31	±1·13	14·1	11·6	12·5	±1·04	±0·90

increased the milk fat percentage. With cow no. 4, the change from the high- to the low-roughage, unsupplemented diet resulted in a decrease in the percentage of fat in the milk but this decrease was not as pronounced as that observed with cows nos. 2 and 3. The addition of tallow or cottonseed oil to the low-roughage diet did not alter the percentage of fat in the milk produced by cow no. 4.

The fatty acid compositions (major components) of the milk fat produced by the cows during the last 4 days of the experimental periods are given in Table 9 and the corresponding yields of the individual fatty acids are given in Table 10. When tallow or cottonseed oil was included in either the low- or high-roughage diets, there was a general tendency for the milk fat to contain lower percentages of the fatty acids up to and including palmitic acid and higher percentages of stearic and oleic acids. These effects of dietary treatments on the fatty acid composition of the milk fat were reflected in the yields of individual fatty acids in the milk fat produced by the cows on the various dietary treatments. For a given concentrate mixture the yields of all the fatty acids were lower in the milk fat of the cows given the low-roughage diets. However, this effect of the level of dietary roughage on the yields of individual milk fatty acids was less pronounced with oleic acid than it was with the other constituent fatty acids.

DISCUSSION

The difference in hay intake between the 2 roughage treatments was greater in expt 1 than it was in expt 2 (Tables 3, 4), yet the yield of milk fat was depressed to a much greater extent by the low-roughage treatments in expt 2 than it was in expt 1 (Tables 5–7). A possible explanation of this observation is that the cows used in expt 1 were at a later stage of lactation than were those used in expt 2. Balch *et al.* (1954) found that the depression in milk-fat percentage resulting from the change from a high- to a low-roughage diet decreased as the stage of lactation advanced. In neither experiment did the addition of tallow or cottonseed oil to the low-roughage diet increase the yield of milk fat. In expt 1, the addition of tallow to the high-roughage diet increased the yield of milk fat throughout the experimental period, but the addition of cottonseed oil to the high-roughage diet increased the yield of milk fat only during the first week of the experimental period. However, these increases in the yield of milk fat are not as great as those reported by Storry, Rook & Hall (1967) who studied the effects of various dietary fats on the yield and composition of the milk of the cow. This difference between our results and those of Storry *et al.* (1967) might be explained by the differences in the fat content of the unsupplemented control diets used in these 2 investigations. In the experiments of Storry *et al.* (1967), the unsupplemented control diet and the fat-supplemented diets, respectively, supplied each cow with about 100 and 400 g fat/day. In our experiment, the unsupplemented control diets and the fat-supplemented diets, respectively, supplied each cow with about 300 and 800 g fat/day. The fact that supplementation of the high-roughage diet with tallow had a more pronounced effect on the yield of milk fat in expt 1 than it had in expt 2 was probably a reflexion of the lower intake of dietary fat, particularly in relation to the output of milk fat in expt 2.

The results given in Table 5 are in agreement with those obtained in earlier investigations (Holland & Buckley, 1918, 1923; Sutton, Brown & Johnston, 1932;

Hilditch & Thompson, 1936; Hill & Palmer, 1938; Williams *et al.* 1939; Hilditch & Jasperson, 1943) which established that the inclusion of vegetable oils such as groundnut, maize, soyabean or linseed oils in the diet of the lactating cow increased the iodine value but decreased the Reichert and Polenske values of the milk fat. Hill & Palmer (1938), Stull, Harland & Davis (1957) and Larsen (1958) all reported that the addition of tallow to the diet of the cow resulted in an increase in the iodine value of the milk fat. The effect of dietary tallow or cottonseed oil on the iodine value of the milk fat observed in expt 1 would appear to be due to the increased concentration of oleic acid in the milk fat produced by cows given these dietary fats (expt 2, Table 9). Hilditch & Thompson (1936) and Hilditch & Jasperson (1943) showed that the increased iodine value of the milk fat of cows given supplementary linseed oil or groundnut oil in the diet was due to an increased percentage of oleic acid in the milk fat. In agreement with the results given in Table 9, Hilditch & Thompson (1936) and Hilditch & Jasperson (1943) showed that this increased percentage of oleic acid was counterbalanced mainly by decreased percentages of the saturated fatty acids containing from 4 to 14 carbon atoms. More recently, Parry, Sampugna & Jensen (1964) found that the inclusion of safflower seed oil in the diet of the cow resulted in the production of milk fat containing markedly lower concentrations of the shorter-chain fatty acids (4:0–14:0) and a markedly higher concentration of oleic acid. Similar effects on the fatty acid composition of the milk fat produced by cows given diets supplemented with vegetable oils rich in the C_{18} dienoic and trienoic fatty acids have been reported by Brown *et al.* (1962), Tove & Mochrie (1963) and Storry *et al.* (1967). The inclusion of cottonseed oil in the diet produced little or no increase in the yield of C_{18} polyunsaturated fatty acids in the milk fat (Table 10). This finding is similar to those of many other investigators (e.g. Brown *et al.* 1962; Tove & Mochrie, 1963; Parry *et al.* 1964). The increased amounts of oleic acid secreted in the milk fat of the cows given dietary cottonseed oil could have arisen by 2 mechanisms. The linoleic acid contained in the cottonseed oil could have been completely hydrogenated by rumen micro-organisms and the resulting stearic acid could have been incorporated into the blood triglycerides and hence taken up by the mammary gland where oleic acid is synthesized by the desaturation of stearic acid (e.g. Annison, Linzell, Fazakerley & Nichols, 1967; Linzell, Annison, Fazakerley & Leng, 1967). On the other hand, the hydrogenation of linoleic acid in the rumen may have been incomplete and some of the resulting octadecenoic acid could have been absorbed into the blood stream and incorporated directly into milk triglycerides in the mammary gland. Presumably, desaturation of stearic acid in the mammary gland played a greater part in increasing the yield of oleic acid in the milk fat of the cows given dietary tallow (containing 28% stearic acid).

In agreement with King & Hemken (1962), the results in Table 9 show that for any given concentrate mixture, the milk fat of the cows on the low-roughage treatment contained higher concentrations of oleic acid and lower concentrations of the shorter-chain fatty acids (4:0–14:0) than did the milk fat of the cows on the high-roughage treatments. This could be explained by the reduced production of acetate in the rumen that is known to occur in cows given low-roughage diets (Rook, 1961). It is difficult to put forward a precise explanation for the general reduction in the yields of the shorter chain fatty acids (4:0–14:0) that occurred in the milk fat when

the cows on the high-roughage treatments were given the diets supplemented with tallow or cottonseed oil. The level of milk fat production in the cow is governed largely by the activities of 2 metabolic processes. In the first of these, acetate and β-hydroxybutyrate are taken up from the blood and are utilized as precursors in the *de novo* synthesis in the mammary gland of the fatty acids from butyric to palmitic acids, which are then incorporated into milk triglycerides (e.g. Ganguly, 1960; Peeters & Lauryssens, 1964; Barry, 1964; Annison *et al.* 1967; Linzell *et al.* 1967). In the second process, triglycerides (containing mainly palmitic, stearic and oleic acids) circulating in the blood stream as chylomicrons and low-density lipoproteins are taken up by the mammary gland. It seems likely that these triglycerides are partially or completely hydrolysed and that the resulting fatty acids are incorporated into new triglyceride molecules in the mammary gland (e.g. Barry, Bartley, Linzell & Robinson, 1963; Patton & McCarthy, 1963; Annison *et al.* 1967). Therefore, any reduction in the supply to the mammary gland of acetate or β-hydroxybutyrate would be expected to reduce the output of the shorter-chain fatty acids in the milk fat. Although the addition of certain highly unsaturated oils (e.g. cod-liver oil) to the diet of the cow is known to reduce the production of acetate in the rumen (Shaw & Ensor, 1959; Nottle & Rook, 1963), it is doubtful whether dietary tallow or cottonseed oil altered the intraruminal synthesis of acetate in the experiments now reported. Brown *et al.* (1962) showed that neither tallow nor cottonseed oil, when incorporated in the diets given to cows at the level of 6% of the concentrate mixture, altered the relative proportions of the volatile fatty acids in the rumen contents. The other possibility is that the increased uptake by the mammary gland of the C_{16} and C_{18} fatty acids inhibited the *de novo* synthesis of the shorter-chain fatty acids from acetate and β-hydroxybutyrate. This possibility has been discussed in some detail by Moore & Steele (1968). One rather anomalous finding was that, in spite of the fact that tallow and cottonseed oil, respectively, contained 30 and 21% palmitic acid, the yield of palmitic acid in the milk fat was not increased when the cows were given the high-fat concentrates. Similar observations were made by Storry *et al.* (1967) who studied the effect on milk fat composition of supplementing the diet of cows with red palm oil (containing 42% palmitic acid).

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Neutral volatiles in Cheddar cheese made aseptically with and without starter culture*

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SUMMARY. Analyses of the neutral volatile components from 3 Cheddar cheese are presented. Two were made aseptically in an aseptic vat, with and without starter culture; the third was made with starter culture in an open vat. Gas–liquid chromatography and mass spectrometry detected the same volatiles in starterless cheese having little or no Cheddar flavour as in cheese made with starter and having a characteristic Cheddar flavour. Methyl disulphide and dimethyl sulphide were the only compounds consistently detected in higher concentrations in the cheese made with starter than in the cheese made without starter. However, using a total trapping technique, it was found that the combination of components recovered from the effluents of the chromatographic columns did not have the cheese-like aroma of the distillate vapours that were injected. Reasons for this are discussed.

The dominant and characteristic portion of the flavour of mature Cheddar cheese is imparted by the odorous volatile constituents of the cheese (Mabbitt & Zielinska, 1956; Jackson, 1958; McGugan, 1963). Work devoted to the isolation and identification of these volatile components has been reviewed by Mabbitt (1961), Marth (1963) and Forss & Patton (1966). Several attempts have been made to duplicate the Cheddar aroma and/or flavour by preparing synthetic mixtures of the identified volatiles or by adding such mixtures to a bland base or to a synthetic base of cheese-like consistency (Day, Bassette & Keeney, 1960; Silverman & Kosikowski, 1953; Walker, 1961). Silverman & Kosikowski (1953) prepared a mixture of amino acids and fatty acids that had a flavour that 'was pleasant, sharp, and had some but not all of the characteristics of typical Cheddar flavor'. Day *et al.* (1960) described a mixture of carbonyls and fatty acids, which included methional and 3-mercaptopropionic acid, as the most cheese-like in aroma of the mixtures tested. They suggested that their failure to produce a complete Cheddar aroma was due to lack of accurate quantitative data as well as to incomplete qualitative data. Walker (1961)

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produced a mild Cheddar flavour typical of a 2–3-month cheese by adding ketones, fatty acids and thioacetamide (as a source of H_2S) to fresh curd and curing at 12·8 °C for 3 weeks. In none of these attempts has the Cheddar aroma been successfully duplicated by mixtures of known components of Cheddar volatiles. Either the correct combination of components had not been found, or there are one or more key aroma components that have not been identified.

Comparison of the volatiles from cheese devoid of Cheddar flavour and from cheese with typical Cheddar flavours should aid in detecting differences in composition that are responsible for the distinct differences in flavour. Cheddar cheese made aseptically without starter culture has a very bland flavour, devoid of characteristics of typical Cheddar (Mabbitt, Chapman & Berridge, 1955). Cheese made with starter develops a normal Cheddar flavour, the flavour of cheese made in an open vat usually being stronger than that made in an 'aseptic' vat (Reiter *et al.* 1967).

This paper reports the results of analyses of cheese made aseptically with and without starter, and a cheese made with starter in an open twin vat.

MATERIALS AND METHODS

Cheese

Samples of Cheddar cheese, made and cured at the National Institute for Research in Dairying (N.I.R.D.), Reading, England, were transported to Ottawa by air. The cheese were made from milk heat-treated at 71·7 °C for 15–20 sec and were cured at 12·8 °C. One cheese (GAL) was made in a closed aseptic vat (Chapman, Mabbitt & Sharpe, 1966) without the addition of starter, δ-gluconic acid lactone providing the necessary acid development (Mabbitt *et al.* 1955; Mabbitt, Chapman & Sharpe, 1959). A second cheese (S/924) was made in the aseptic vat, using a single-strain starter (*Streptococcus cremoris* 924). A third cheese (S/924 Control) was the same as the second except that it was made in an open twin vat. Further details of manufacture, and bacteriological and free fatty acid analyses of cheese made by the same procedures are given by Reiter *et al* (1967). The cheese were shipped when 20 weeks (GAL) and 24 weeks of age (S/924 and S/924 Control), and analysed within 8 days of receipt.

Flavour evaluation

The Cheddar flavour intensities of the cheese were assessed by taste panels at Reading (9 tasters) and Ottawa (8 tasters). The balanced incomplete block method described by Elliott & Beckett (1959) was used. The 3 samples in each block were ranked 1, 2 and 3 in order of increasing Cheddar flavour intensity. A fourth cheese (X) for which no analytical data are presented in this paper was also included in the taste panel assessment. The taste panel also provided data for use in the study of microbial flora and free fatty acid conducted at N.I.R.D. The rank totals were as follows:

	GAL	S/924	S/924 Control	X
Reading	30	62	68	56
Ottawa	25	55	61	51

Completely consistent ranking by all panel members would have yielded the following rank totals:

Reading	27	63	81	45
Ottawa	24	56	72	40

Estimates of concordance by Bradley's (1955) method were 0·519 ($P < 0·001$) for the Reading data and 0·591 ($P < 0·001$) for the Ottawa data.

Isolation of cheese volatiles

One hundred grams of cheese were packed into 50-ml stainless steel centrifuge tubes, sealed with closures with neoprene washers, and centrifuged for 20 min at 25000 *g*. Immediately after centrifuging, the tubes were opened and the fat was decanted into a previously evacuated (0·001 torr, Pirani gauge) distillation apparatus (Fig. 1). About 60 % of the cheese fat is separated by centrifuging. After carefully

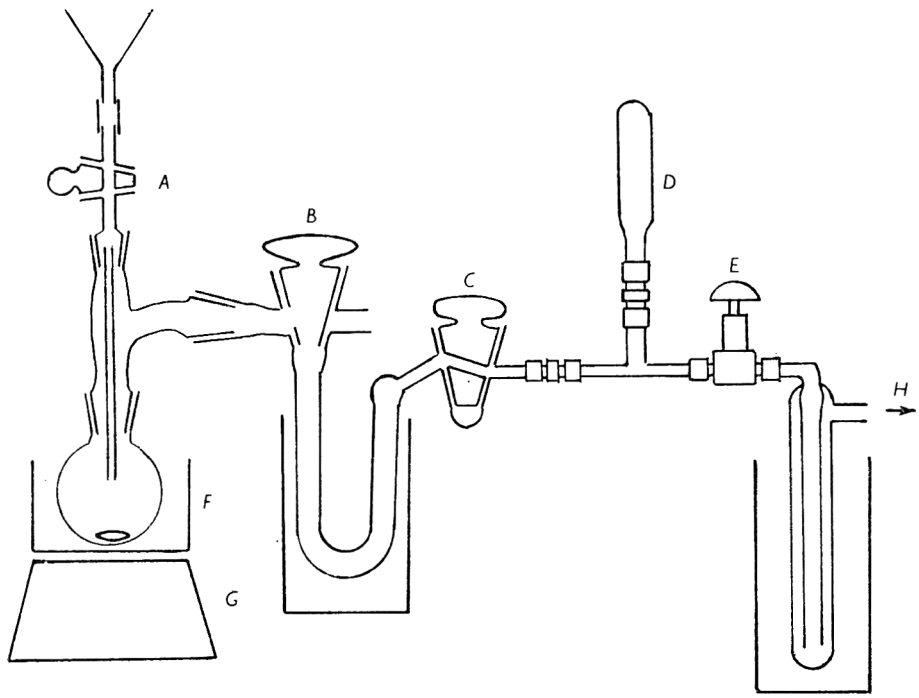

Fig. 1. Apparatus for vacuum distillation of volatiles from cheese fat. *A*, stopcock to admit cheese fat; *B*, *C*, stopcocks to isolate the distillate trap immersed in liquid nitrogen; *D*, Pirani gauge; *E*, valve to isolate system from vacuum pump; *F*, water bath; *G*, magnetic stirrer-heater; *H*, connexion of isolation cold-trap to mechanical vacuum pump.

pumping off the non-condensable gases through the liquid–nitrogen trap B–C to a pressure of 0·012 torr, water at 40 °C was added to the water bath. Distillation was continued for a further 2 h at 40 °C with vigorous stirring. On completion of the distillation, stopcocks B and C were closed and the trap was removed from the system. The trap was fitted with a 'helium-type' balloon on one end and a serum cap on the other. Then the trap was warmed to the sampling temperature and the

vacuum was broken with nitrogen from the balloon. The volume of the trap, including a 10-ml inflation of the balloon, was 135 ml.

A separate distillate was prepared for each chromatographic run.

Gas chromatography

Gas chromatograms of the cheese distillates were obtained using 2 different columns in an F and M Model 810 gas chromatograph with a flame-ionization detector.

Column A was 650 ft × 0·02 in. I.D. stainless steel tubing coated with Carbowax 1540. The inlet helium pressure was 20 psi; the flow rate with the column at 50 °C was 3·5 ml/min, as measured with a bubble flow meter at the column outlet. The column temperature was programmed at 1 °C/min after 20 min at 50 °C. At the injection port, a short length of the column was brought outside the oven and formed into a 5-turn coil of 5-cm diam. The coil was cooled in an isopentane bath at −145 °C. A 10-ml vapour sample was slowly injected onto the column. It was necessary to sample the distillate vapours at −10 °C to avoid blocking the trapping coil with ice.

Column B was 5 ft × 0·125 in O.D. stainless steel tubing packed with 100–120 mesh Porapak Q, the whole of the column being inside the oven. The inlet helium pressure was 25 psi; the flow rate at 50 °C was 22 ml/min. The column temperature was programmed from 50 °C at 11 °C/min for 8 min, then at 2·2 °C/min to 215 °C. A 10-ml vapour sample was injected. The distillate vapours were sampled at 25 °C, reducing the degree of discrimination against the higher boiling compounds that occurred on sampling at −10 °C.

Mass spectrometry of the gas chromatograph effluent

Mass spectra were obtained of components eluted from Column A via a Watson–Biemann helium diffusion device (Watson & Biemann, 1965) directly into a Bendix time-of-flight mass spectrometer. For qualitative identification of eluted components by mass spectrometry, a larger sample of distillate was chromatographed. A different procedure was used to prepare the distillate, in order to reduce interference in the mass spectrometer caused by water and carbon dioxide. Distillates from the fat from 400-g cheese samples were obtained by distilling for 4 h at 40 °C. Carbon dioxide was removed from the distillate by vacuum sublimation at −135 °C. The distillate then was fractionated by vacuum sublimation from the distillate trap at −60 °C into a second U-tube trap (24 ml) in liquid nitrogen. Sublimation was allowed to continue until the pressure in the system had dropped to 0·012 torr. This produced a sublimate which retained a cheese-like aroma similar to that of the original distillate but which was relatively free from water. It was possible to sample the sublimate vapours at 40 °C and inject 15 ml without plugging the −145 °C-trapping coil in the GLC column.

Mass spectra were not obtained from the effluent from column B, due to the limited capacity of the helium diffusion unit.

RESULTS

Tables 1 and 2 show the retention times and peak areas of components from the 3 cheese samples chromatographed on columns A and B, respectively. The identities of components in Table 1 are based on data both from GLC and from mass spectra. Those in Table 2 are based on retention times of known samples of compounds whose presence in the distillates had been established by retention and mass spectral data from column A. The data on peak areas of the major components are summarized in Table 3. The gas chromatograms are shown in Fig. 2.

Table 1. *Cheese volatiles chromatographed on* 650 *ft* × 0·02 *in.* I.D. *Carbowax* 1540 *column (Col. A)*

Cheese	GAL		S/924		S/924 Control	
Date of manufacture	28. vii. 66		29. vi. 66		29. vi. 66	
Age, days	137		167		168	
Component	Retention time, min	Peak area, cm^2	Retention time, min	Peak area, cm^2	Retention time, min	Peak area, cm^2
Dimethyl sulphide	14·3	Tr*	14·2	0·39	14·2	0·26
C_8 Hydrocarbon	14·6	Tr	14·6	Tr	14·7	Tr
Acetone	15·1	9·0	15·1	30·3	15·2	5·7
Ethyl acetate	16·5	0·39	16·5	Tr	16·6	0·26
Butanone	17·0	0·71	17·0	0·97	17·0	77·4
?	17·4	Tr	17·5	0·52	—	—
2-Propanol	17·8	1·0	17·8	0·90	17·8	4·3
Ethanol	18·2	63·0	18·6	16·1	18·6	43·9
?	19·5	Tr	19·6	Tr	—	—
2-Pentanone	19·9	9·7	20·0	12·3	20·2	14·8
?	20·8	Tr	—	—	—	—
Terpene + $CHCl_3$	22·2	Tr	22·1	Tr	—	—
2-Butanol	—	—	—	—	22·8	14·8
Ethyl butyrate	23·1	Tr	23·3	Tr	23·6	Tr
Toluene	23·5	6·2	23·8	6·2	24·2	5·8
n-Propanol	—	—	—	—	25·6	0·64
Hexanone	27·2	0·39	27·6	Tr	28·0	Tr
2-Methyl-1-propanol	28·1	Tr	28·8	Tr	—	—
2-Pentanol	30·3	Tr	—	—	31·7	0·39
Xylene	32·8	Tr	33·5	Tr	34·0	Tr
n-Butanol	—	—	36·7	Tr	37·6	Tr
2-Heptanone	38·0	4·6	38·8	0·45	39·7	0·26

* Tr = trace.

The components shown on the column A chromatograms (Fig. 2) emerging before 14 min are C_5–C_7 hydrocarbons. The mass spectra also revealed the presence of Freon 12 and carbon disulphide in this area. Other minor components that were detected by mass spectrometry but not evident in the column A chromatograms are: ethyl formate and carbon tetrachloride, between acetone and ethyl acetate; methyl disulphide, just before hexanone, and a trace of a C_6 ester following toluene. Methyl disulphide was just detectable in the spectrum from the GAL cheese but was appreciably stronger in the spectra from the other 2 cheese.

Table 2. *Cheese volatiles chromatographed on 5 ft × 0·125 in. O.D. Porapak Q (Col. B)*

Cheese	GAL		S/924		S/924 Control	
Age, days	141		173		174	
Component	Retention time, min	Peak area, cm²	Retention time, min	Peak area, cm²	Retention time, min	Peak area, cm²
Methanol	6·0	Tr	6·0	Tr	—	—
?	7·5	Tr	7·5	Tr	—	—
Ethanol	9·0	155	9·1	21·3	9·0	45·2
?	10·7	Tr	—	—	—	—
Acetone	11·7	11·6	11·6	31·0	11·8	5·0
2-Propanol	12·3	Tr	12·2	Tr	12·3	7·1
Dimethyl sulphide (?)	—	—	12·7	Tr	12·9	Tr
n-Propanol + hydrocarbon	14·2	1·9	14·1	1·7	14·1	2·9
Hydrocarbon (?)	15·1	3·3	15·0	1·5	14·6	
?	16·1	Tr	—	—	15·0	
?	—	—	16·5	Tr	—	—
?	17·4	Tr	—	—	—	—
Butanone	18·4	1·2	18·2	1·4	18·2	75·5
Ethyl acetate + 2-Butanol	19·6	0·58	19·6	Tr	19·3	40·6
2-Methyl-1-propanol	20·7	0·64	20·7	0·64	—	—
n-Butanol	22·6	4·5	22·6	Tr	23·0	0·39
?	24·9	0·39	24·9	1·4	25·1	Tr
2-Pentanone	26·3	41·9	26·4	23·2	26·6	12·3
?	28·1	1·5	—	—	28·4	2·6
C_5 Alcohol	30·3	2·8	30·4	1·5	31·0	Tr
Hydrocarbon (?)	31·6	Tr	31·6	Tr	—	—
Toluene	33·2	11·6	33·1	10·3	33·4	9·0
Hexanone ?	35·5	5·5	—	—	—	—
Ethyl butyrate	36·4		36·5	0·71	36·8	1·8
2-Heptanone	45·0	28·8	45·4	3·0	45·3	2·9

Table 3. *Summary of quantitative GLC data for the major components of Cheddar volatiles*

Cheese	GAL		S/924		S/924 Control	
Column	A	B	A	B	A	B
Component	Relative peak areas, cm²					
Acetone	9·0	12	30	31	5·7	5·0
Ethyl acetate	0·4	0·6	Tr*	Tr	0·3	—†
Butanone	0·7	1·2	1·0	1·4	77	76
2-Propanol	1·0	Tr	0·9	Tr	4·3	7·1
Ethanol	63	155	16	21	44	45
2-Pentanone	9·7	42	12	23	15	12
2-Butanol	—	—	—	—	15	41†
Toluene	6·2	12	6·2	10	5·8	9·0
2-Heptanone	4·6	26	0·4	3·0	0·3	2·9

* Tr = trace.

† Ethyl acetate and 2-butanol emerge together from column B.

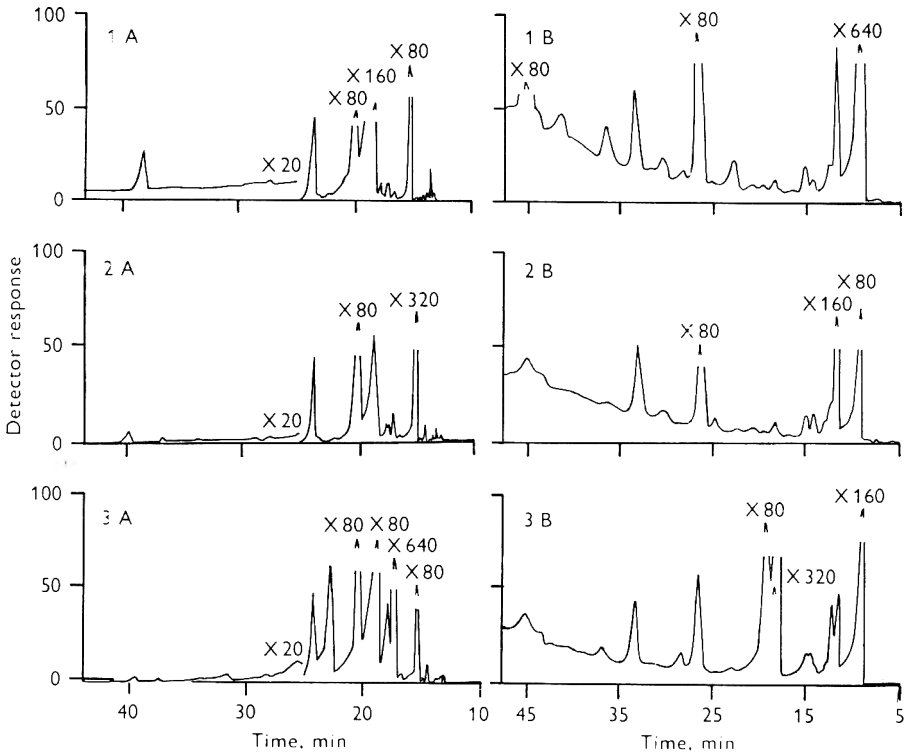

Fig. 2. Gas chromatograms of cheese distillates chromatographed on columns A and B. Sample 1, GAL; 2, S/924; 3, S/924 control.

DISCUSSION AND CONCLUSIONS

The taste panel results indicated that the GAL cheese possessed little typical Cheddar flavour as compared to the 2 starter cheese. However, among the major components listed in Table 3 there are no consistent quantitative differences between the starter cheese and the GAL cheese that correspond to the differences in Cheddar flavour intensities. Sample S/924 is higher in acetone than is GAL, but S/924 Control is lower. The control cheese is high in butanone, 2-propanol and 2-butanol but S/924 contains about the same amounts of these compounds as does GAL. The GAL cheese is higher in ethanol and 2-heptanone than is the starter cheese. Among the trace components detected, dimethyl sulphide and methyl disulphide were the only compounds present in higher concentrations in both of the starter cheese than in the GAL cheese.

It is recognized that the taste panel rankings were based on the total flavour of the cheese, whereas the analytical data were obtained from the volatile components only. However, since the aroma provides the dominant and characteristic portion of the flavour, any influence of the background broth-like taste is not likely to invalidate the conclusions.

The failure to detect any volatile components that appeared to be directly responsible for the cheese-like aroma of the distillate suggested the following possibilities:

one or more components of the distillate that are essential to Cheddar aroma were retained on the columns, either completely or partially; one or more components were altered (decomposed, isomerized) by passage through the columns, thereby altering their aroma characteristics; the combination of sample size and detector sensitivity was too low to detect components that are essential to the cheese aroma. Compounds that do not emerge from the columns under the conditions used are free fatty acids. Patton (1963) concluded that the volatile free fatty acids were the closest approach to Cheddar aroma provided by any compound or class of compounds, but did not, in themselves, completely simulate the aroma. Accepting this conclusion, our data still fail to indicate the compounds that modify the fatty acids to produce the Cheddar aroma. Day & Libbey (1964) detected 130 components in a Cheddar distillate. They were unable to identify 84 of these, most of which were trace components. It is possible that some of these trace components are essential components of the aroma that were not detected by our methods due to limited sample size or choice of chromatographic conditions. However, since Day & Libbey did not determine that material with a cheese-like aroma could be recovered from the column effluent, consideration of the significance of the unidentified trace components is purely speculative.

To determine if our failure to account for the Cheddar aroma was due to low detector sensitivity, the total material eluted from the column during chromatography of a sample of cheese distillate was condensed in a U-tube trap cooled in liquid nitrogen. It was found that the material recovered did not have the cheese-like aroma of the sample of distillate vapour that had been injected into the gas chromatograph. This proved to be true with both columns used in this work, and with several others, including a column recommended by Klein (1967) for the resolution of free fatty acids (6 % FFAP on Poropak T). The only columns of those tested, from which material with a cheese-like aroma could be recovered, were packed with Teflon, or a silanized support, coated with Apiezon. Work on this aspect of the problem is continuing, and will be reported in a separate paper.

The data presented in this paper show that there are numerous volatile components in cheese made without starter and devoid of Cheddar flavour. With the exceptions of traces of dimethyl sulphide and methyl disulphide, no compound was shown to be present in higher concentration in both of the starter cheese than in the GAL cheese; a few compounds were present in higher concentration in one or the other of the starter cheese, but not in both. This suggests that these compounds are not of major significance in the characteristic flavour of Cheddar. The 2 sulphur compounds do emerge from the columns, yet the total condensible material recovered from a chromatographic run, with the columns used, did not possess the cheese-like aroma of the samples of distillate vapour that were injected on to the columns. The obvious conclusion is that in this work, and possibly in that of others, one or more components essential to Cheddar cheese aroma have not been eluted from the columns used and hence remain unidentified. It is evident that instrumental analyses of flavours should be supported by evidence that the flavour has not been altered by the methods used in the analyses.

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Studies on the laboratory soiling of milking equipment

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Summary. A laboratory method has been developed for studying the action of chemical disinfectants on milking equipment using a wet film and both metal and rubber surfaces. It was shown that milking machines may be soiled as effectively with fresh milk of low bacterial content as with aged milk of high bacterial content. When a single heat treatment preceded the soiling with milk and subsequent treatment with detergent and chemical disinfectant, a build-up of bacteria began on the third day. However, when the surfaces were heat treated daily 18 h before soiling, followed by cleaning and disinfection, no build-up occurred. These findings support the hypothesis that contamination of milk results from bacteria developing on the milking equipment and not merely from those added to it at each milking. The sites of bacterial contamination were shown to be the under surfaces of lid gaskets and the joints of the short milk tubes and the metal clawpiece nipples.

Some understanding of how milk contact surfaces become contaminated and of how micro-organisms develop on contaminated surfaces is necessary in order to simulate in the laboratory the condition of dairy equipment on the farm. To carry out laboratory soiling of equipment it is necessary to consider: (*a*) the nature of the surface to be soiled; (*b*) the nature of the soiling medium and (*c*) the deposition of the soiling medium on the equipment and the nature of the soiling film.

The surfaces to be soiled should be similar to those used in practice; with machine milking equipment both metal and rubber surfaces are involved. These have been used separately by various workers in laboratory trials on the efficiency of disinfectants, but we are not aware of work in which both metal and rubber surfaces have been used. Furthermore, the surfaces should be treated and kept under conditions similar to those in the farm dairy.

In earlier work the soiling medium has normally been milk heavily infected with bacteria, or a 'standard' soiling medium consisting of milk and other adhesive materials (Guiteras & Shapiro, 1946). Such soiling media, however, do not occur naturally.

The nature of the soiling film and the method of depositing it are of great importance. Dried and aged films, as used by many workers, can occur under farm dairy conditions but are not usual. On the other hand, a wet film deposited on a clean

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surface would appear too unstable for test purposes. Repeated soiling and cleaning over a period of days or weeks would more closely approach practical conditions.

The farm trial is the ultimate method of assessing the action and efficiency of chemical disinfectants on milk contact surfaces. However, such trials are subject to many uncontrollable variables which mask the effect of planned differences in experimental procedure. Laboratory trials can be more closely controlled, and provided they simulate farm conditions it should be possible to determine with greater precision the factors affecting the rate of build-up of contamination on milking equipment and hence devise more effective and practical ways of reducing or preventing it. The present work was undertaken with the object of developing a suitable laboratory method.

Following the completion of studies comparing aged and fresh raw milk as the soiling medium and of the efficiency of heat and chemical disinfection (Part I), further trials were made to determine the exact location of the high levels of bacterial contamination which developed on the milking machine units used as the test-surfaces (Part II).

Part I

Materials and methods

Test surfaces

Suspended type milking machine units, each comprised of a stainless steel bucket, lid and a teat cup cluster, were fitted with new natural or synthetic rubber teat liners (inflations).

Soiling medium

Raw milk aged at 77 °F (25 °C) until the standard plate count reached *c.* 2×10^7 bacteria/ml was used for the first experiment. Subsequently, fresh raw milk having a count of *c.* 5×10^3/ml was used, as indicated.

Soiling the equipment

Each unit was soiled in turn by drawing 2 gal milk by vacuum through the cluster and into the bucket, the same milk being used for all the units. The soiled equipment was left at 24 °C for 30–60 min before cleaning and disinfection. The milk contact surfaces were still wet after this time.

Cleaning and disinfection

(1) *Separate detergent and disinfectant treatment*

Directly after soiling

(*a*) *Pre-rinse.* Each unit was vacuum-rinsed with 2 gal clean water at 60 °F (15·6 °C).

(*b*) *Detergent wash.* For each unit, 2 gal solution at 46·1 °C containing 0·25% (w/v) of alkaline detergent (Pennsan MC-3, Pennsalt Chemicals of Canada Ltd, Oakville, Ontario) was drawn from a pail by vacuum through the cluster into the machine bucket. Two-thirds of this quantity was drawn through each cluster into the machine bucket and the mouthpiece of the clusters allowed to soak in the pail submerged in the detergent solution for 2 min. The solution in the machine bucket was swirled a few times and discarded.

(*c*) *Clean rinse.* Each unit was vacuum-rinsed with 2 gal clean water at 26·7 °C which was swirled in the bucket and then discarded. The units were left at 23·9 °C for 4–5 h before testing.

Directly before testing

(*d*) *Sanitizing rinse.* Each unit was vacuum-rinsed with 2 gal sodium hypochlorite solution at 15·6 °C containing 300 ppm. available chlorine. The solution was swirled in the unit bucket and discarded and the equipment drained and tested within 10 min.

(2) *Combined detergent-disinfectant treatment*

Directly after soiling

(*a*) *Pre-rinse.* As in (1 *a*) above.

(*b*) *Detergent-disinfectant wash.* For each unit 2 gal solution containing 0·25 % (w/v) alkaline detergent (see (1 *b*) above) and 300 ppm. available chlorine was used to wash each bucket and cluster as in (1 *b*) above.

(*c*) *Final chlorinated rinse.* Sodium hypochlorite solution containing 75 ppm. available chlorine was used as in (1 *d*) above but immediately after (2 *b*). The units were left at 23·9 °C for 4–5 h before testing.

Heat treatment

At the beginning of each experiment and during one of the experiments, as indicated, the buckets, lids and clusters were heat-treated by immersion in water which was brought to boiling point by steam injection and boiled for 10 min.

Bacteriological examination of the milking machine units

After cleaning and chemical disinfection each complete unit (bucket, lid and cluster) was rinsed with 500 ml of a solution of pH 7·2 containing, per litre, 40 mg KH_2PO_4, 500 mg $Na_2S_2O_3$ $5H_2O$, 4g Asolectin (Associated Concentrates Inc., Woodside, New York) and 10 g Tween 80 (Atlas Powder Co., Wilmington, Del.) as recommended by American Public Health Association (1960). The rinsing procedure was the pulsating rinse first used by Claydon (1953) and modified by Bačić & Clegg (1967). The rinses were plated on standard plate count agar and incubated for 2 days at 32 °C.

RESULTS

Previous work in this laboratory had suggested that after milking equipment had been heat-treated it was not possible to achieve an artificial build-up of bacteria on the equipment surface before about 3 days even after soiling with aged milk. To confirm this finding 6 machine buckets and clusters were heat-treated and soiled with aged milk and then cleaned and disinfected with hypochlorite. To find out if the method of cleaning and disinfection had any influence on the build-up of bacteria, combined and separate cleaning and disinfection processes were used as detailed in the methods section.

Table 1 compares the results obtained using the 2 methods of disinfection. They show the same general phenomena in both cases. This experiment was not intended to be a comparison of the 2 methods of treatment because the treatments were not

duplicated in all machine units. Thus, the fact that the combined treatment gave results inferior to those with the separate treatment is of no significance. The difference between counts for the individual units is of interest. Unit G gave low counts in a number of other experiments, though the results in Table 2 are an exception.

It is noteworthy that no build-up of bacteria occurred until about the third day after heat treatment even though the equipment was soiled with milk having a count of $> 2 \times 10^7$ bacteria/ml. This phenomenon has been noted in a number of other experiments done for different purposes and not reported here.

Table 1. *The effects of repeated experimental soiling of milking machine units with aged milk containing c.* 2×10^7 *bacteria/ml, followed by cleaning with an alkaline detergent and chemical disinfection with sodium hypochlorite*

Days after heat treatment of unit	Colony count/ml of bacteriological rinses from individual units following: Combined cleaning and disinfection. units			Separate cleaning and disinfection. units		
	A	C	D	H	E	G
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	20000	500	70000	600	7000	0
4	2500	1000	240000	500	3000	0
5	25000	1000	220000	2500	50	0
8	3000	9400	70000	8000	100	0
9	20000	20000	70000	500	1000	50
10	12000	2500	30000	20000	200	100

In the light of the findings in Table 1 it was planned to investigate whether build-up of bacteria could be prevented by daily heat treatment *before* the equipment was soiled. As some earlier experiments had suggested that the rate of bacterial build-up on equipment surfaces was not greatly affected by the bacterial content of the soiling medium, this question also was investigated.

Fresh milk with a count of *c.* 5×10^3 bacteria/ml was used as the soiling medium. All the units were heat treated before soiling commenced and half were heat treated daily thereafter. All were cleaned and disinfected daily with sodium hypochlorite. As can be seen in Table 2 the treatments were repeated so that between-unit differences did not influence the results. Control units were included in the experiment and these were tested before cleaning and disinfection.

Again the phenomenon of a build-up of bacteria after the third day is apparent on units which received only the initial heat treatment. With those units which were heated daily before chemical sterilization, no build-up occurred during the period of the experiment.

Direct comparison between Tables 1 and 2 of results for units A and C shows that there was little difference in the rate of bacterial build-up whether the soiling medium contained few or many bacteria.

Table 2. *The effects of daily heat treatment of milking machine units before experimental soiling with fresh milk containing* 5×10^3 *bacteria/ml and of combined cleaning and chemical disinfection with an alkaline detergent and sodium hypochlorite*

	Colony count/ml given by bacteriological rinses from individual units											
	Heat treated daily before soiling. units			Heat treated once only at beginning of experiment. units			Heat treated daily before soiling. units			Heat treated once only at beginning of experiment. units			Control after pre-rinse only. No heat treatment, cleaning or disinfection
Day after first soiling	A	B	C	F	G	H	F	G	H	A	B	C	D
1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	920	220	300	0	0	0	300	3000	4000	7000
6	5	10	6	9500	60500	5200	0	0	0	32000	80	800	70000
7	0	0	0	3000	100000	38000	0	0	0	200000	4000	40000	280000
8	0	0	0	9500	180000	110000	0	0	0	80000	32000	120000	500000
9	—	—	—	—	—	—	0	0	0	30000	230000	8000	600000

PART II

After completion of the experiments described in Part I an attempt was made to determine more precisely the location of bacteria recovered in rinses of the complete units which had revealed counts of up to 70000/ml of rinse ($3{\cdot}5 \times 10^7$ bacteria/unit) 3 days after heat disinfection.

It was noted that the rubber gaskets of the milking machine bucket lids showed signs of deterioration; the rubber had hardened and tended to shrink away from the rims of the lids. Traces of what appeared to be milk residues were present between the gasket and the lid. These gaskets had not been renewed nor were they removed at any time during the experiments described in Part I. Consequently, the experiments were repeated and some of the experimental procedures were modified to see whether the high rinse counts were associated with the lid gaskets.

MATERIALS AND METHODS

The milking machine units were similar to those used previously. The metal buckets and lids of 6 units were descaled, washed, rinsed and steamed for 10 min. Four units (J, K, L and M) were fitted with new synthetic rubber liners and new natural rubber lid gaskets all of which had been treated in water at 85–90 °C for 5 min. The remaining units (N and O) were fitted with used synthetic and natural rubber liners and used natural rubber lid gaskets.

After assembly the units were soiled as before. Fresh raw milk having a standard plate count of $3\text{–}6 \times 10^4$ bacteria/ml was collected once a week and was used daily for 5 days. It was refrigerated immediately after use but its colony count increased considerably, on occasion to 1×10^6/ml, after 5 days.

A separate cleaning and disinfection procedure was used as described in Part I except that the disinfectant rinse contained 100 ppm. available chlorine. The lid gaskets of some units as indicated later received extra treatment. After the detergent wash and again after the disinfectant rinse the gaskets were removed and soaked in the solution; in addition, the gasket seatings were swabbed with solution thus preventing the accumulation of milk residues. Initially the bacteriological rinse solution was similar to that used in Part I. Subsequently and for the most part, quarter strength Ringer's solution containing 0·05 % (w/v) sodium thiosulphate was used, because this made it unnecessary to rinse the equipment with cold water after the bacteriological rinse.

The rinse procedure was similar except that during the 2-min pulsation of the solution in the teat cups and before it was released into the bucket a small quantity of solution was withdrawn with a sterile pipette from each teat cup to test the level of contamination present on the liners. Occasionally, before rinsing a complete unit, the bucket was rinsed separately using 500 ml rinse solution; 10 ml was withdrawn for testing and the same solution was used for rinsing the unit by the normal test procedure. Bacteriological tests were made as before.

RESULTS

Daily rinse counts for units K, J, L and M, fitted with new rubbers, are detailed in Table 3. Removing the lid gaskets from L and M for additional cleaning reduced residual contamination as compared with K and J, but counts on all 4 units were variable with up to 700/ml of rinse for L and M and up to 18700/ml for K and J. They were considerably lower, however, than those shown in Tables 1 and 2. It is evident that removal of the gaskets did not eliminate contamination.

Rinses of the rubber liners and of the stainless steel buckets which were tested separately at intervals throughout the experiment showed that these surfaces contributed very few micro-organisms; counts for liners ranged from 0 to 8 and for buckets from 0 to 3/ml of rinse. Clearly, there was some source of contamination in addition to the lid gasket seatings at some site between the liner barrels and the bucket in each of the 2 complete units.

Table 3. *The build-up of contamination in milking machine units fitted with new liners and lid gaskets, and the effect of removing lid gaskets for additional cleaning and disinfection*

Days after descaling and heat-treatment	Colony count/ml of rinse of unit			
	J	K	L	M
			(Lid gaskets removed)	
3	0	0	0	36
4	0	20	0	0
5	84	80	3	17
6	71	113	62	10
7	4800	111	14	62
8	89	620	226	13
9	305	1120	29	36
10	56	690	35	50
11	2390	1160	570	460
12	370	1490	600	102
13	3200	1860	290	132
14	2700	2160	710	—
15	400	220	9	3
16	260	610	7	74
17	2230	5400	580	232
18	880	440	77	116
19	340	1420	570	370
20	103	310	15	17
21	7300	18700	304	14
22	910	1270	153	93
23	150	1570	148	31
24	580	770	1*	2*
25	8400	13	1*	0*
Geometric mean	210	270	30	22

* Short milk tubes removed from clawpiece and brushed.

During the bacteriological rinse procedure the short milk tubes from the liners were flexed to occlude the tube and the rubber was forced away from the metal nipple of the clawpiece. It seemed possible that in this way bacteria were exposed or released from these sites during the rinse procedure. They were presumably trapped at some time during the soiling of the units.

On the last 2 days of the experiment, therefore, the short milk tubes of units L and M were detached from the metal nipples during cleaning, and were brushed with detergent solution and finally rinsed with hypochlorite solution. The treatment was effective in reducing the contamination as shown by the rinse counts for the complete units L and M for days 24 and 25 (Table 3).

Table 4 shows results for units N and O which had been fitted with old rubber components. To simulate more closely the conditions of the experiments in Part I the units were soiled and washed for 4 days to allow milk residues to accumulate under the lid gaskets; the lids with the gaskets *in situ* and the remainder of the equipment were then heat treated as described above.

Table 4. *The build-up of contamination in milking machine units fitted with used liners and lid gaskets and the effect of removing the lid gasket of unit N for additional cleaning and disinfection*

Days after heat treatment	Colony count/ml of rinse of unit	
	N	O
0	2	0
1	266	2
2	241	29
3	13000	121
4	30000	9120
5	40000	40800
6	44*	16200
7	540*	43000
8	280*	40000

* Lid gasket removed for additional cleaning and disinfection.

The daily soiling and washing procedure was repeated and rinses were taken in the same way as for the other 4 units. Within 5 days of heat treatment both units showed high rinse counts (40000/ml of rinse or 20×10^6 bacteria/unit) comparable to the build-up of bacteria shown in Tables 1 and 2. The liners and buckets were again remarkably free from contamination and counts for both natural and synthetic rubber liners and for the buckets did not exceed 3/ml of rinse.

On the 6th day after heat treatment the lid gasket of unit N was removed, washed and disinfected separately and this additional treatment reduced dramatically the unit rinse count in comparison with unit O (Table 4). Similar results were obtained on days 7 and 8, after which the experiment was brought to an end.

DISCUSSION

Most of the earlier work with artificial soiling of surfaces has been done with dried milk films (cf. Weber & Black, 1948; Hoy & Clegg, 1953; Garvie & Clarke, 1955; Goetchius & Botwright, 1950). While this approach has given valuable information, the soiling of surfaces with milk, followed by drying, did not simulate the conditions of soiling on the farm. Accordingly it was considered that the application of milk to a surface, followed immediately by rinsing off the wet film, cleaning and disinfection without brushing and repeating the whole procedure daily would approach more closely to farm practice.

In this work, it was at first thought that heavily infected milk would be necessary in order to ensure the survival of micro-organisms which could be detected after disinfection. However, earlier work (Bačić, Jackson & Clegg, 1967) showed that numbers of bacteria from bulk milk bore no relation to the numbers of bacteria from the cow's udder. It was concluded that the micro-organisms must have come from the utensils and therefore it was reasoned that fresh milk should be as effective a soiling agent as aged milk. This proved to be the case and explains how a build-up of bacteria can occur on farm dairy utensils. The finding that it takes 2 or 3 days for a build-up of bacteria to occur which can be detected by rinsing, regardless of the numbers of micro-organisms in the soiling medium, supports our hypothesis that contaminating bacteria develop on the equipment and are not merely added to the equipment at each milking.

The value of heat treatment as a safeguard for chemical sterilization has been demonstrated (Clegg, 1955). Further evidence of the necessity for such heat treatment is shown in Tables 1 and 2. After effective heat disinfection the build-up of bacteria is delayed for about 3 days. Consequently, with daily heat treatment a build-up should never occur and confirmatory evidence of this is presented in Table 2. We have further evidence that this situation can continue for at least 20 days and there seems no reason why it should not continue indefinitely.

Using a flush-washing technique it has been shown that with chemical disinfection build-up of contamination is restricted to certain sites in the milking equipment, notably the gasket on the bucket lid. It is apparent that milk is able to penetrate the area between the gasket and its seating on the lid, and that repeated soiling promotes bacterial multiplication. Bacteriological rinses can dislodge micro-organisms from such a site. Where the rubber is in poor physical condition and does not fit tightly, build-up is more rapid and perhaps contamination is more readily released by the rinsing procedure than when the rubber is new (Table 4). The results in Table 3 illustrate the benefit, in terms of lower bacterial counts, of rubber in good physical condition. The day-to-day variation in rinse counts probably reflects the variable amount of movement or disturbance of the gaskets during the testing procedure.

It is of considerable interest that both rubber and metal surfaces which had no joints or crevices (teat cup liners and buckets) remained free from contamination for at least a month when disinfected with sodium hypochlorite using a flush-washing technique and without brushing.

The results help to explain the failure of chemical disinfection of milking equipment over a period of time. Even where surfaces are in good condition and free from residues, washing by hand may fail if dismantling of certain components, e.g. short milk tubes from clawpiece, is infrequent or inadequate. With pipeline milking equipment, cleaning and disinfection by circulating solutions at low temperatures will permit bacterial multiplication in certain sites, e.g. in badly fitting joints and on the under surface of gaskets, where traces of milk can accumulate (Cousins, 1967). In such circumstances heat treatment is essential.

It seems reasonable to suppose that where milkstone or a film has developed on the surfaces (Clegg, 1962) or where deterioration causing crevices and imperfections has occurred so that any micro-organisms present are protected from contact with

the chemical disinfectant, a build-up of contamination may occur more generally on the equipment surfaces.

The technique of repetitive soiling, cleaning and disinfection should be of value as a test procedure for evaluating detergents and disinfectants. It simulates contamination of equipment under farm conditions and, as testing can be carried out over an extended period, the method could be used to determine the 'breakdown point' of any cleaning and disinfecting routine. It is important, however, that hazards inherent in the construction and the components of the equipment used, are recognized and taken into account.

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A pilot plant for the removal of cationic fission products from milk

I. Design and construction

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Summary. A pilot plant with a capacity of 2300 l./5 h day for the removal of cationic fission products from milk is described. The process involves the acidification of the milk with citric acid to pH 5·25 and its passage through an ion exchange resin charged with the ions of Ca, K, Na and Mg in the same proportions as those in which they occur in milk. The effluent milk is neutralized with potassium hydroxide. At the end of the day the plant and resin bed are washed and sterilized.

Two resin beds are provided and are used on alternate days, one being washed and regenerated while the other is in use. Regeneration is carried out with a solution which removes radioactive cations and restores the resin bed to its original ionic composition.

Bacteriological tests show that the method of cleaning both plant and resin bed is satisfactory.

Conclusions are drawn as to a suitable design for a larger scale plant.

The debris from nuclear weapons contains a number of radioactive nuclides which eventually contaminate foodstuffs and especially milk. From the point of view of possible dangers to health the most important of these nuclides are the isotopes of strontium (^{90}Sr, half-life 28 years) and caesium (^{137}Cs, half-life 30 years).

Strontium follows much the same biochemical pathways as calcium and is therefore deposited in bone, from which it is removed very slowly by metabolic processes. The irradiation of bone tissue and marrow by ^{90}Sr may cause bone tumours and leukaemia. Although ^{137}Cs is not bone-seeking it is distributed throughout the soft tissues and thus also irradiates the bone marrow.

It must, however, be noted that the total dose of radiation received by the population from fallout resulting from tests carried out up to the end of 1965 is extremely small. The Medical Research Council (1966) calculate that the total dose delivered to the bone marrow of individuals born before the testing of nuclear weapons began and surviving to the year 2000, will be only about the same as that delivered by the natural background in 1·2 years. About half of the dose from fallout would be due to ingested ^{90}Sr and ^{137}Cs of which milk is an important source, especially for young children. In case weapon testing should be resumed and concentrations of these nuclides in milk become very much greater, techniques for their removal have been

explored in this country and elsewhere. This paper describes the construction and operation of a pilot plant designed to remove at least 90 % of ^{90}Sr from milk by a process of ion exchange. Preliminary accounts of this plant and of various aspects of its performance have already been published (Glascock, 1965; Glascock & Bryant, 1966).

Methods of decontamination

Ion exchange resins

Reports of work on the removal of radioactive ionic contaminants from milk first appeared in the literature in 1959. At this time the ^{90}Sr content of milk was low but was increasing. The country-wide average was 6·6 pC/l. in 1957 rising to a maximum of 40·5 pC/l. in the period July 1963–June 1964 (Agricultural Research Council, 1966).

Cosslett & Watts (1959) reported experiments on the use of Zeo-Karb 225 (a sulphonated polystyrene resin) for the removal of radiostrontium previously added to milk *in vitro*. They found that they could remove 87–98 % of the radioactivity with the resin in the calcium form and 95–98 % of the natural calcium with the resin in the sodium form. They concluded that treatment with the resin in the calcium form would remove radiostrontium introduced *in vivo* with the same efficiency (95–98 %) but this in fact was not so as later work has shown.

Migicovsky (1959) found that batch treatment with Dowex resins in the calcium form removed up to 97 % of ^{89}Sr from milk to which it had been added *in vitro* but replaced substantial proportions of the sodium and potassium at the same time. By using a resin saturated with the ions of calcium, potassium and sodium in the same proportions as those in which they occur in milk he was able to remove 85–90 % of the radiostrontium without appreciable change in the ionic composition of the treated milk. He obtained much the same efficiency of removal of radioactivity on a mixture of normal cow's milk with guinea-pig's milk containing ^{89}Sr introduced *in vivo*. This result was not confirmed, however, by Murthy, Masurovsky & Campbell (1961) who found that only 45–50 % of the radioactivity was removed when 25 resin bed volumes of cow's milk containing ^{89}Sr introduced *in vivo* and at its natural pH were passed through a mixed ion bed. Acidification of the milk to pH 5·4, however, resulted in an increase in the efficiency of removal to 90–95%.

Acidification of the milk before treatment and the use of a resin bed charged with a suitable mixture of cations is the basis of the U.S. patent granted in 1962 (Murthy, Campbell, Masurovsky & Edmondson, 1962) and of the process operated on a pilot plant scale (450 l./h) at Beltsville and on a large scale (5700 l./h) at Springfield, Missouri. (Edmondson *et al.* 1962; Sadler, Walter, Hanrahan & Edmondson, 1967; Producers Creamery Company, 1965; Sparling *et al.* 1967). Although the patent provided for neutralization of the milk either by the addition of alkali or by the use of an anion exchange resin, neutralization with alkali was used in both these plants. Edmondson (1964), however, has described a laboratory apparatus with a capacity of 750 ml/min designed for continuous operation in which an anion exchange resin is used for neutralization and simultaneous removal of ^{131}I. The resin beds are pumped hydraulically round the system in such a way that part is in contact with the milk and part with the salt solution which removes the radioactive contaminants. The

Chemical Separations Corporation (1963) have described a pilot plant version of the same apparatus with a capacity of 450 l./h.

Other methods

Tri-calcium phosphate added to milk will remove 95 % of ^{90}Sr introduced *in vitro* if the mixture is held at 70 °C for 5 min (Silverman, Ghosh & Belcher, 1963). This, of course, is essentially a process of ion exchange but as 30 % of the milk protein and 50 % of the calcium are removed at the same time (Edmondson, Landgrebe, Sadler & Walter, 1965) the method is not feasible for large-scale use.

Electrodialysis can be used either to effect a radical alteration in the ionic composition of milk or just to remove radioactive cations. The milk is passed between pairs of cation-selective membranes which separate it from 2 salt solutions. Under the influence of an imposed electric field, cations pass from the milk into one salt solution and are replaced by cations from the other. For the removal of ^{90}Sr, the milk must first be acidified to pH 5·2 when an efficiency of removal of 90–99 % for both ^{90}Sr and ^{137}Cs is obtained. A model plant with a capacity of 22 l./h was constructed for the U.S. Office of Civil Defence by Ionics Ltd. (Greatorex & Glass, 1965) who also offer larger plants for sale or hire.

The plant at the National Institute for Research in Dairying, Shinfield

As a consequence of a decision announced by the Minister of Science on 27 February 1963 a plant was erected at Shinfield for studies on the removal of radioactive cationic contaminants from milk essentially by the process described and patented by Murthy *et al.* (1962). The object of the work was to confirm the results obtained by American workers, to provide first-hand experience of the process and to improve it if possible. The patent (Murthy *et al.* 1962) describes the process as carried out on a laboratory scale (50 ml/min) and the design of a pilot plant capable of treating 450–550 l./h was much facilitated by the visit of one of us (R. F. G.) in 1961 to see the pilot plant in operation at Beltsville, Washington, D.C., by a description of it published in 1962 by Edmondson *et al.* and by personal correspondence with Dr Edmondson and his colleagues. In particular, a schematic diagram of a proposed large-scale plant (5500 l./h) supplied by the United States Department of Agriculture in September 1962 was of great use. A royalty-free licence to operate the process for experimental purposes was granted by the United States Government.

Since this plant was built reports have appeared on the automated pilot plant installed at Beltsville (Sadler *et al.* 1967), and on the large-scale plant operated by the Producers Creamery Company at Springfield, Missouri (Producers Creamery Company, 1965; Sparling *et al.* 1967; Heinemann *et al.* 1967). The automated pilot plant was very similar in size to the Shinfield plant, but the authors did not say how many times it was run nor whether any attempt was made to detect corrosion or deterioration of components or of the resin bed. The large-scale plant, which was also largely automated, had a capacity of 45400 l./8-h day and was run experimentally 9 times in all, 4 times on skim-milk and 5 times on whole milk.

Summary of Specification

General

The plant at Shinfield is constructed in stainless steel to British Standards (1956) B.S. 1449 grade En 58B except for the resin columns and associated fittings. Since these parts come into contact with regenerating solution, stainless steel of grade En 58J is used. Pipe-fittings conform to Standard FIL/IDF 14:1960 of the International Dairy Federation. Provision is made for conventional in-place cleaning of the whole plant, which generally is constructed to the usual standard for dairy equipment. The pipe through which regenerating solution is pumped is constructed in rigid PVC, mostly solvent-welded but provided with screw connexions where easy removal of parts is necessary.

Tanks

For untreated milk. This is an insulated tank with a capacity of 2500 l. and fitted with an electrically driven agitator.

For treated milk. Two tanks are provided, each with a capacity of 4500–5000 l. One is connected to drain and is fitted with a solenoid-operated valve actuated through a time-switch on the control panel.

For detergent solution. The tank has a capacity of 550–600 l. and is provided with a loose fitting lid.

For regenerating solution. Five tanks are provided, all lined with plastics and each with a capacity of 800 l. One, used for the preparation of the concentrated stock solution, is fitted with an electrically driven agitator and is connected to a supply of hot water.

For acid and alkali. Each of these tanks has a capacity of 45 l. They are provided with loose fitting lids and are mounted above the metering pumps.

Pumps

For milk. These are centrifugal pumps of sanitary design and have a capacity of 900 l./h against a head of 1 atm. They were supplied by A.P.V. Co. Ltd. and by Gascoignes (Reading) Ltd.

For detergent solution. This is a centrifugal pump constructed of stainless steel with a capacity of 11000 l./h against a pressure of 3·5 atm. It was supplied by A.P.V. Co. Ltd.

For regenerating solution. This is a positive displacement pump with a drive of variable speed giving a maximum output of 900 l./h against 0·75 atm pressure. The parts coming into contact with the solution are made of plastics. It was supplied by Mono Pumps Ltd., London, E.C.1.

For acid and alkali. Two metering pumps (supplied by Bran and Leubbe (Great Britain) Ltd., Market Harborough) of maximum output 150 ml/min are used. They are of constant speed and the stroke can be adjusted manually.

Flowmeters

All the flowmeters were supplied by Rotameter Manufacturing Co. (Croydon) Ltd.

Injection nozzles

The injection nozzles for acid and alkali each consist of a stainless steel tube of internal diam. 6 mm with one end blanked off. Two holes, 0·8 mm in diam., have been drilled diametrically opposite each other, 8 mm from the closed end. The nozzles are connected to the acid and alkali metering pumps and are inserted in the line by means of a suitable tee.

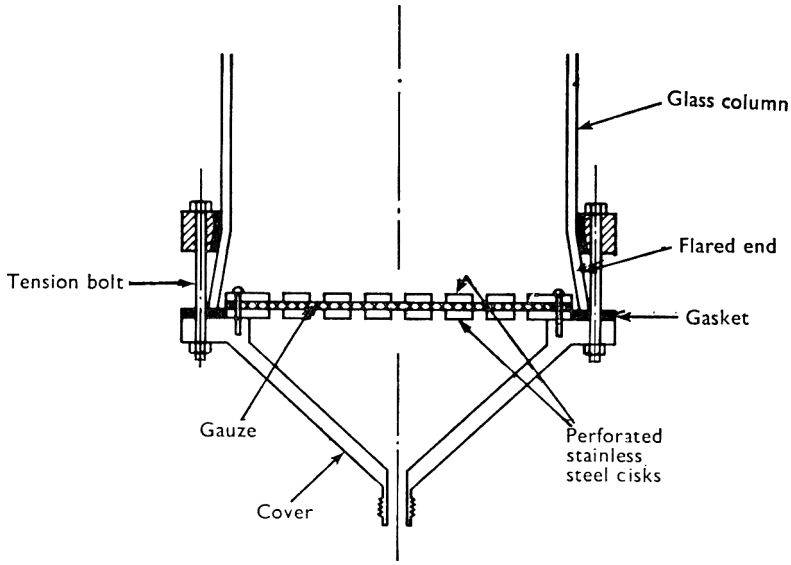

Fig. 1. Base of column with conical cover showing details of support for resin.

Resin columns

One column is of borosilicate glass of internal diam. 30 cm and length 205 cm with flared ends and was supplied by Q.V.F. Ltd., Stoke-on-Trent. Each end is fitted with a conical flanged cover made of 16-gauge stainless steel and secured to the column by means of a backing flange, food quality rubber gasket and tension bolts. The resin bed is supported on a stainless steel gauze, mesh size 60, held between a pair of stainless steel plates, 4·5 mm thick, and containing 25 holes of diam. 6 mm distributed evenly over the surface. This unit fits inside the end of the column and is screwed to the conical cover as shown in Fig. 1. At the upper end of the column there is a similar gauze, reinforced with 2 pairs of 3-mm stainless steel cross-wires, and held in place with a single annular plate bolted to the cover.

The other column is of similar size and shape; the lower part, 140 cm long, is constructed of stainless steel, polished inside, while the upper part, 65 cm long, is made of glass. The column was so constructed because it was found necessary to lengthen both columns in the course of experiments and a glass section was therefore added to both. This in no way interferes with the testing of stainless steel as a material for construction of the column and obviates the necessity of providing an observation port.

Resin

Each column is charged with 75 l. of resin which about half fills it. The resin is Zeo-Karb 225 (SRC 13) (Permutit Co. Ltd.) a sulphonated polystyrene with 8 % cross linkages and of particle size 16–52 mesh.

pH *Sensing equipment*

All the pH-sensing electrodes are Pye-Ingold. Addition of acid is controlled with a Standard Glass Electrode no. 201 coupled to an Industrial Argenthal Reference Electrode no. 365 separately inserted into the line; and the addition of alkali is controlled with an Industrial combined Glass and Reference Electrode no. 465. Both the reference electrode and the combined electrodes are in electrical contact with the milk through a salt bridge and glass sinter. If necessary, both types of electrode can be pressurized to several atmospheres, to prevent milk being driven into the sinter. Each pH electrode system is connected to a Pye Dynacap pH-meter. This is in turn coupled to a Honeywell Chart Recorder which contains a device for ringing an alarm when the pH passes outside preset limits.

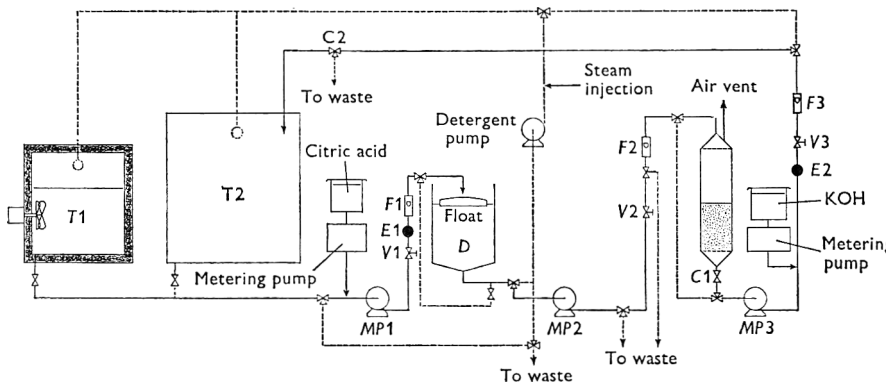

Fig. 2. Plant for removal of ^{90}Sr from milk. *C* 1, 2, cocks: *D*, delay tank; *E* 1, 2, electrodes; *F* 1, 2, 3, flowmeters; *MP* 1, 2, 3, milk pumps; *T* 1, untreated-milk tank; *T* 2, treated-milk tank; *V*, valves.

Plant design and operation

Figure 2 is a diagram of the plant in its final form. The day's charge of raw milk (about 2300 l.) at a temperature of 4–5 °C is received into milk tank T 1, the agitator is started to prevent separation of the cream during processing and the milk is pumped into tank *D* by means of milk pump *MP* 1. The rate of discharge into this tank is adjusted to the required value (540 l./h) by valve *V* 1 and flowmeter *F* 1. Citric acid (2·5 N) solution is injected into the milk stream just before it enters milk pump *MP* 1 by means of the metering pump which is adjusted manually so that the pH of the milk, as detected by the electrode *E* 1, is 5·25. The chart recorder is set so that the alarm rings if the pH varies by more than ± 0·03 units from this value. If that happens either the rate of acid injection or the rate of milk flow is corrected according to which has changed.

Tank *D* was originally a detergent tank but later was also used as a delay tank,

and serves to hold the milk for an hour between acidification and application to the resin column. To prevent mixing and thus to ensure that all the milk is held acid for about the same period the tank is provided with a stainless steel float upon which the incoming milk impinges.

Only one resin column is shown in the diagram but in the plant the 2 columns are so arranged that either can be brought into use by readjustment of 'key pieces'. The resin bed, charged with the principal milk ions, has been previously washed with a dilute solution of hypochlorite and left just covered with this solution.

Milk is pumped from the delay tank D to the resin column by means of milk pump $MP2$, and valve $V2$ is adjusted so that flowmeter $F2$ gives the same reading as flowmeter $F1$ thus ensuring that once the delay tank is full, the level in it remains constant until milk tank $T1$ is empty. The air-vent at the top of the column is kept open until the space above the resin is full of milk. It is then closed and cock $C1$ at the foot of the column is opened. The milk and water mixture which first emerges is run to waste until the electrode $E2$ indicates a pH of 5·3. The metering pump which injects 2·5 N-KOH into the milk stream is started and the rate of delivery is adjusted so that the pH of the milk is restored to 6·7. The milk is then directed by means of cock 2 to the treated milk tank $T2$. Since the pH of the milk is less critical after neutralization than after acidification, the chart recorder is set so that the alarm bell rings when the pH passes outside the range 6·65–6·75. If this should happen an adjustment is made to the metering pump. Towards the end of the run when tank D is empty, milk pump $MP2$ is no longer in operation. The residual milk in the column and pipe work is then pumped to tank $T2$ with milk pump $MP3$ and its flowrate adjusted, with valve $V3$ and flowmeter $F3$, so that the pH remains constant.

Only one tank for treated milk is shown in the diagram but on the plant there are two. Normal milk is collected in one and milk to which ^{85}Sr has been added for experimental purposes is collected in the other. The normal milk is sold for pig feeding but the milk still containing traces of ^{85}Sr is discharged into the sewer at a time of day and at a rate specified by the local authority.

Cleaning

The in-place cleaning of all pumps, tanks and pipes follows conventional practice of commercial dairies. The dotted lines in Figure 2 indicate the supplementary pipe-work required for this purpose.

The resin columns are cleaned by the following procedure, all solutions passing upwards at a rate sufficient to bring the whole of the resin bed into suspension. The bed is washed with cold water from the mains for 45 min. Alkaline detergent solution at 80 °C containing hypochlorite to give 100 ppm. available chlorine is then circulated for 20 min and the bed completely drained. It is washed with water at 60 °C for 5 min, drained and 90 l. of 0·5 N-nitric acid at 60 °C circulated for 20 min. It is finally washed with cold water from the mains. The bed is left covered with water and drained immediately before regeneration.

Regeneration

The regenerating solution used to remove radiostrontium from the resin and to charge it with the required mixture of ions is of the composition recommended by Edmondson *et al.* (1962) and contains per litre: calcium chloride (dihydrate), 53·5 g; potassium chloride, 23·1 g; sodium chloride, 8·5 g and magnesium chloride (hexahydrate), 15·1 g. The chemicals are of the cheapest available commercial grade. The suitability of this grade has been examined and is reported on by Glascock & Bryant (1968).

The technique of regeneration used is that recommended by Edmondson (1964). It permits the re-use of three-quarters of the regenerating solution at each cycle, thus economizing in chemicals which represent the most expensive part of the process.

A stock solution of 635 l. of the salt solution at 3 times the above concentration is prepared in a tank provided with a mechanical stirrer and a hot water supply. This concentration is very nearly the maximum obtainable. For charging a new resin bed with the mixed ions this stock solution is then diluted 3-fold (1900 l. or 25 bed volumes) and distributed between tanks 1–4. The contents of tank 4 are then pumped through the resin bed, first by upward-flow to displace the air and then by downward-flow, and run to waste. When tank 4 is empty, the contents of tank 3 are pumped through the column and collected in tank 4. This process is continued, the contents of each tank moving one place up, until finally tank 1 is empty and is replenished with fresh regenerating solution.

This same procedure is used when the resin bed has been used for the treatment of milk. Thus, when the plant is in continual use the solution in tank 4 has been used 3 times, that in tank 3 twice and that in tank 2 once. The efficiency of the procedure for the removal of radiostrontium from the column has been investigated and is reported on by Glascock & Bryant (1968).

At the end of the regeneration procedure, the resin bed is washed for about 30 min with cold mains water and is then left until the next day covered with water. Just before use it is given a pre-process rinse with cold hypochlorite solution containing 50 ppm. available chlorine.

Plant development

The above description is of the plant in its final form. A number of modifications were made in the course of development, however, and these are of some interest.

Control of pH

The plant at Shinfield, like the pilot plant at Beltsville (Sadler *et al.* 1967) and the large-scale plant at Springfield (Sparling *et al.* 1967), originally incorporated equipment for the close automatic control of pH at both the acidification and neutralization stages. This was effected by regulation of the stroke of the metering pump with equipment coupled to the pH meter. Some difficulty was experienced with this equipment and it was soon realized that it was, in fact, unnecessarily complicated for the task to be performed. The buffering capacity of the milk remains constant during a day's run and hence there is no variation in the acid demand for a given change of pH. The pumping rates of milk and acid are now adjusted manually as

already described and the pH sensing equipment used only to ring an alarm when the pH needs adjustment. This conclusion was also reached by the operators of the large-scale plant at Springfield and is included in their list of possible changes of design (Producers Creamery Company, 1965, p. 90).

Type and positioning of electrodes

As originally designed the acid was injected at a point after milk pump *MP*1 and a Pye-Ingold combined glass and reference electrode was used to measure the pH. This electrode was inserted before valve *V*1, which is in a region of high pressure. It was often found that the pH recorded on the plant meter was 0·2 unit lower than the true value and that when this occurred the Pye-Ingold electrode had a clot on the glass sinter through which the salt bridge and the milk were in electrical contact.

It was thought that the clot was due either to an accidental over-shooting of the acid during the initial adjustment of the metering pump, to incomplete mixing of the acid with the milk resulting in local precipitation, or to milk being driven into the sinter in spite of the fact that the electrode was pressurized. Three modifications were therefore made simultaneously: first the point of injection of acid was moved to a position just before the entrance to milk pump *MP*1 thus ensuring good mixing in the pump rotor chamber; second the electrode was inserted on the low pressure side of valve *V*1 and thirdly the type of electrode was changed (see specification). This was necessary because the sinter on the type originally used was not only extremely small (about 1 mm diam.) but was also slightly below the surface of the glass body and consequently very difficult to clean. The sinter of the type of electrode now used has a diam. of about 2·5 mm and is mounted flush with the surface. When these changes had been made no further difficulty was encountered in pH control.

Filter

A large-capacity duplex cloth filter was originally installed between milk pump *MP*1 and the delay tank *D*. It was found to collect so little solid material that its use was discontinued.

Support for the resin-bed

A stainless steel gauze reinforced with a pair of cross wires was originally used to support the resin bed. It was found, however, that during cleaning by upward-flow it was impossible to bring the whole of the resin bed into suspension. At rates of flow below that which would pack the resin against the upper gauze there was always a stagnant zone round the lower part of the column walls. The gauze was therefore mounted in the perforated plates already described. This resulted in jets of water being produced, especially round the periphery, thus bringing the whole resin bed into suspension.

Cleaning

Edmondson *et al.* (1962) reported that adequate cleaning of the resin beds of their first pilot plant was achieved by a preliminary rinsing followed by washing with a non-ionic detergent by upward-flow. Much the same procedures were also used by these same workers on their automated plant (Sadler *et al.* 1967) and by

Sparling *et al.* (1967) on the full-scale plant at Springfield, with the addition of an 'air sparge' to agitate the suspended resin bed. In the plant at Shinfield, however, this washing procedure was found to be inadequate. After the first rinse it was found that the resin bed contained a number of large lumps some 10–15 cm in diam. which were not broken up by a non-ionic detergent. Some improvement was obtained by the use of an ionic detergent although this resulted in the displacement of some of the calcium ions from the resin into the alkaline solution making it turbid. This was not objectionable, however, because the ionic composition of the resin was restored at the regeneration stage. Even with this detergent some smaller lumps remained and experiment showed that they broke up immediately on treatment with dilute acid. The nitric acid wash was therefore introduced into the cleaning programme. It was found to be completely successful and has caused no detectable corrosion of the parts with which it comes in contact. Nitric acid solutions are, of course, occasionally used in dairy plant to remove milk residues.

PERFORMANCE

The radiochemical efficiency of the process and the properties of the treated milk are dealt with in a separate communication, and we report here only on the mechanical performance of the plant and on the efficiency of the cleaning process.

In its final form, as specified in this paper, the plant has been run 120 times and during this period no abnormal wear or corrosion of parts has been observed. The repeated washing and regeneration of the resin has produced no detectable physical deterioration and losses due to washing out of fine particles have been negligible. As is reported elsewhere (Glascock & Bryant, 1968) there was also no deterioration in radiochemical efficiency.

Cleaning

When the plant was first operated it was found that the regenerating solution was subject to contamination by an unidentified organism which in turn contaminated the resin bed. Thus, whereas fresh regenerating solution contained only 100 organisms/ml, once-used solution contained 250000 and twice-used solution contained 2700000 organisms/ml. The addition of hypochlorite to this solution to a concentration of 50 ppm. available chlorine completely prevented the growth of this organism and in no way impaired the regeneration process.

No significant increase in bacterial count of the milk has ever been found to result from its passage through the plant, and rinse water both from pipework and resin has always been found to contain less than 10 organisms/ml, which is acceptable for dairy plant.

Design of a large-scale plant

This plant was constructed for the purpose of providing experience of the process and information which would help in the design of a large-scale plant. It is unlikely that such a plant would have a capacity of less than 5000 l./h and much of the equipment would therefore have to be scaled up by at least a factor of 10. Although much of the control would no doubt be automated there would be no important

changes of design. Thus, acid and alkali injection systems would be similar to those on the pilot plant and effected by means of metering pumps whose output can be varied by manual adjustment. Similar pH electrodes, meters and recording-alarm systems would also be used.

A 10-fold increase in the size of the resin bed would probably be best achieved by increasing the diameter to about 100 cm and retaining the present bed-depth of 100 cm since this depth has been found to be effective both on the pilot plant and laboratory scale. Columns of this diameter could quite easily be made in stainless steel, and this has, in fact, been done by the constructors of the plant at Springfield. Although it was thought, when the plant at Shinfield was first designed, that a full-scale plant would have stainless steel columns and although the Americans have in fact used them, our experience suggests that glass columns would be preferable. Even on an automated plant it would be useful for the operator to be able to inspect the resin bed, especially during cleaning, and glass inspection ports might well provide crevices which would be difficult to clean effectively. The widest glass column at present available has a diam. of 60 cm and if this was used the depth of the resin bed would need to be 250 cm instead of 100 cm. Whether this changed geometry would still necessitate a column of twice that height for it to be possible to bring the whole bed into suspension by upwashing, would have to be ascertained by experiment.

The plant was constructed to our specification by T. Giusti and Son Ltd. Our thanks are due to the following: Mr F. P. Temme and Mr A. G. Perkin for help in the installation of the plant; Mr A. H. Place and Mr G. A. Payne for help in its operation; and Dr C. M. Cousins under whose supervision the bacteriological tests were carried out. We also thank Mr J. L. Cole of the Milk Marketing Board for organizing the supply of milk.

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A pilot plant for the removal of cationic fission products from milk

II. Efficiency of the process and composition of the product

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SUMMARY. As previously described, the process consists essentially of the passage of milk at pH 5·2–5·25 through an ion exchange resin charged with a suitable mixture of the ions of Ca, K, Na and Mg.

When citric acid was used for reduction of the pH and 30 resin bed volumes (r.b.v.) were treated, the concentration of residual ^{85}Sr was reduced to 2–4 % whether it had been introduced *in vivo* or *in vitro*. There was no evidence that repeated use of the resin bed resulted in diminished radiochemical efficiency.

About the same efficiency of removal of ^{133}Ba was obtained but this efficiency was achieved with ^{137}Cs only if not more than 15 r.b.v. were treated. If 30 r.b.v. were treated the concentration of residual ^{137}Cs was as much as 30 %.

When hydrochloric acid was substituted for citric acid the residual concentration of ^{85}Sr was 11 %.

Analyses of milks processed after adjustment of pH with either citric or hydrochloric acid showed the products to be satisfactory. The only important constituent lost was thiamine. Concentrations of heavy metals remained well below maximum values. Although the flavour of milk was detectably changed by the treatment it was still acceptable.

The results are discussed in relation to the problem of achieving maximum decontamination and to the design of a large scale plant.

INTRODUCTION

A pilot plant erected in this Institute for the removal of cationic fission products from milk has been described in a preceding paper (Glascock, Hall, Suffolk & Bryant, 1968). The process used was essentially that described and patented by Murthy, Campbell, Masurovsky & Edmondson (1962). Reports by American workers of applications of the technique on both a pilot-plant scale (450 l./h) (Sadler, Walter, Hanrahan & Edmondson, 1967) and a large scale (5500 l./h) (Producers Creamery Company, 1965; Heinemann *et al.* 1967; Isaacks, Hazzard, Barth, Fooks & Edmondson, 1967) have appeared since this work was started. Our main objective in setting up the plant was to provide first-hand experience of the process, and we now report our findings on the radiochemical efficiency of the process and on the composition of the treated milk. They agree in many respects, though not all, with those obtained

by American workers. Preliminary accounts of some of the results have already been published (Glascock, 1965; Glascock & Bryant, 1966).

The concentration of stable strontium in milk produced on farms in Berkshire, England, is about 300 μg/l. (Bryant, Chamberlain, Morgan & Spicer, 1957). Radiostrontium introduced into the milk *in vivo* becomes inseparably mixed with this stable strontium. Whether the radiostrontium is ^{90}Sr derived from fallout or ^{85}Sr added for experimental purposes, the amount of nuclide so added is negligible in weight. Thus, ^{90}Sr at the average concentration of 9·3 pc/l. found in milk in Britain during July–September 1967 (Agricultural Research Council, 1968) corresponds to only 0·07 pg/l., and ^{85}Sr at a concentration of 0·2 μc/l. as used for testing the performance of the plant corresponds to only 8·5 pg/l. The problem of removing ^{90}Sr from milk is, from the chemical point of view, therefore, one of removing the 300 μg/l. of stable strontium. Similar considerations apply also to ^{137}Cs although the concentration of the stable element in milk is not known precisely. From indirect evidence it appears to be in the region of 1–10 μg/l. compared with which the current concentration of ^{137}Cs (17 pc/l. or 0·2 pg/l.) is also negligible.

According to Edmondson (1964) about 80 % of the total strontium in milk is in the casein complex and 20 % in the free ionic state in the serum. All of that in the serum and part of that in the casein complex is accessible to ion exchange resins so that at normal pH about 45 % is exchangeable. As the pH is lowered the proportion of strontium in the serum increases until at pH 5·2 about 98·5 % of the total is exchangeable.

Because of this distribution of stable strontium in milk, radiostrontium added *in vitro* equilibrates only slowly at normal pH. By comparing the efficiency of removal of ^{85}Sr from milk at pH 5·4 by ion exchange resins after allowing the nuclide added *in vitro* to equilibrate for various periods of time, Edmondson, Keefer, Douglas, Harris & Dodson (1963) found that mixing of the radioactive isotope with the stable element continued for at least 120 h. Even then, when treated with ion exchange resins at pH values above 5·8, the efficiency of removal of ^{85}Sr was appreciably higher when it had been introduced *in vitro* than when it had been introduced *in vivo*. With diminishing pH, however, the efficiencies of removal differed less until at pH 5·2 they were virtually identical. Hence, in the present work, in which the pH of the milk was always reduced to 5·2–5·3, most of the tests of radiochemical efficiency were done on milk containing ^{85}Sr added *in vitro*. Furthermore, since Edmondson *et al.* (1963) found that there was very little difference in the efficiency of removal of ^{85}Sr from milks allowed to equilibrate with the nuclide for 72 and 120 h, the shorter period was always used. Alkali metals exist only in the ionic state in milk and ^{137}Cs should therefore be removed by ion exchange resins with the same efficiency, whether originally introduced *in vivo* or *in vitro*.

EXPERIMENTAL

Materials and methods

^{85}Sr, ^{137}Cs and ^{133}Ba were obtained from the Radiochemical Centre, Amersham. Milk at 4–5 °C was obtained direct from the farms. Citric acid was B.P. quality; potassium hydroxide was 'Puriss' flake (L. R. B. Pearce Ltd, London, W.C. 1) and

the chlorides of calcium, potassium, sodium and magnesium were the cheapest available industrial grades.

Introduction of radionuclides into milk in vitro

About 500 μc of the nuclide was added to 20 l. of milk which was stored at 4–5°. ^{85}Sr and ^{133}Ba were allowed to equilibrate for 72 h and ^{137}Cs for 16 h. This milk was then added to a day's charge of about 2300 l. immediately after its delivery into the untreated milk tank and stirred until the mixture was of uniform specific activity.

Introduction of radionuclides into milk in vivo

A Friesian cow, in late lactation and yielding about 14 l./day, was used for the preparation of milk containing ^{85}Sr or ^{137}Cs. In 2 separate experiments, 0·49 and 0·41 mc ^{85}Sr were obtained in the milk collected during the 3 days which followed intravenous injections of 5 mc $^{85}SrCl_2$.

Milk containing 10·6 μc ^{137}Cs, sufficient for an experiment with a laboratory column, was obtained during the 3 days following the intravenous injection of 100 μc of $^{137}CsCl$.

Measurement of radioactivity

The specific radioactivity of milk was determined with an Ekco scintillation counter connected to a type 1700 EHT scaler unit manufactured by Isotope Developments Limited, Aldermaston, Berkshire. The milk was contained in a specially designed beaker of capacity 450 ml which fitted closely over the 25 mm thallium-activated sodium iodide crystal of the scintillation counter. The efficiency of counting was 1·96 % for ^{85}Sr, 0·95 % for ^{137}Cs and 3·1 % for ^{133}Ba.

Samples of milk for analysis

Suitable 'tees' were provided on the plant for the withdrawal of samples of milk during processing. Acidified untreated milk was obtained from a point near the top of the resin columns; treated milk before neutralization was obtained from a drain cock on milk pump *MP*3 (Fig. 2, Glascock *et al.* 1968) and treated neutralized milk was withdrawn from a point near tank *T* 2.

All the 30 samples of each kind of milk (unprocessed milk and milk treated with hydrochloric or citric acids during processing) which were prepared for nutritional experiments (R. Braude, R. F. Glascock, M. J. Newport & J. W. G. Porter, unpublished) were used for the determination of solids-not-fat (SNF) and fat. The rest of the analyses were carried out on 4 composite samples of each kind of milk. These were prepared by mixing equal volumes of the 7 or 8 separate samples used in each of the nutritional experiments.

ANALYTICAL METHODS

In the analysis of the main samples of milk Mg was determined by the method of Willis (1960) after incineration of the samples as described by Smith & McAllan (1966). Calcium was also determined in the ash by dissolving it in acid, separating

calcium as oxalate and titrating with $KMnO_4$. In the experiments on the variation of mineral content with volume of milk treated (Fig. 3 (*d*)) Ca and Mg were determined volumetrically as described by Ntailianas & Whitney (1964).

For the determination of soluble calcium and phosphorus milk was subjected to ultra-filtration through regenerated cellulose ('Visking') tubing.

Phosphorus was determined spectrophotometrically in milk ash by the standard vanadium phosphomolybdate method; potassium and sodium by flame photometry after precipitation and removal of proteins; chloride according to the method of the British Standards Institution (1963); citrate by the spectrophotometric method of Marier & Boulet (1958) and total nitrogen by the Kjeldahl method.

Lead, copper and iron were determined spectrophotometrically as their complexes with diphenylthiocarbazone, sodium diethyldithiocarbamate and thioglycollic acid, respectively. Arsenic and antimony were determined by the Gutzeit method.

Stable strontium was separated as the nitrate, after addition of ^{85}Sr as a recovery indicator, and determined by flame photometry.

Fat was determined by the Gerber method (British Standards Institution, 1955) and its fatty acid composition by gas liquid chromatography as described by de-Man (1964).

The enzymic clotting time was measured after the addition of rennin (0·02 %) to the milk sample and incubation at 37 °C.

The freezing point was measured by the Hortvet method (British Standards Institution, 1959).

Vitamins were determined as follows: thiamine was assayed with *Lactobacillus viridescens*, as described by Deibel, Evans & Niven (1957), and vitamin B_6 with *Kloeckera brevis* by the method of Barton-Wright (1946), as modified by Gregory (1959). Vitamin B_{12} was assayed with *Lactobacillus leichmannii* as described by Gregory (1954). Riboflavin, nicotinic acid and biotin were assayed by standard microbiological procedures (Ford, Gregory, Porter & Thompson, 1953; Chapman *et al.* 1957). Folic acid was assayed with *Lactobacillus casei* as described by Ford (1967).

Fat-soluble vitamins (A and D and α-tocopherol) were determined as described by Thompson, Henry & Kon (1964) except that α-tocopherol was purified on Florisil instead of on Decalso F.

RADIOCHEMICAL RESULTS

Preliminary experiments with a laboratory column

When the plant was designed American workers advised a column of diam. 30 cm and a bed depth of 74 cm for a flow rate of milk of 0·125 r.b.v./min (L. F. Edmondson, personal communication).

Experiments on milk containing ^{85}Sr introduced *in vitro* and acidified to pH 5·35 with citric acid were therefore carried out at a flow rate of 0·125 r.b.v./min on a laboratory column 70 cm in height and 15 mm in diam. As shown in Fig. 1 (*a*), the total residual activity was only 1 % in 25 r.b.v. and 1·5 % in 30 r.b.v. of treated milk. These experiments suggest that a depth of resin bed of 70 cm would be adequate on the plant although in fact a deeper bed came to be used in order to make possible the treatment of the desired quantity of milk in the course of a day. The slight diminution in concentration of residual ^{85}Sr in the effluent milk between the start of the

experiment and the passage of 20 r.b.v. was probably due to the effect of keeping the milk acid during that time (see later).

Preliminary experiments on the plant: determination of optimum bed volume

Preliminary experiments were carried out on the plant with milk containing ^{85}Sr introduced *in vitro*. When milk acidified with citric acid to a pH meter reading of 5·35 was passed through a resin bed 60 l. in volume, the concentration of residual ^{85}Sr remained virtually constant at 4–6 % until 900 l. (15 r.b.v.) had been treated. The concentration then increased smoothly until, by the time 1800 l. (30 r.b.v.) had been treated, it had reached 10 %. At this point the residual ^{85}Sr in all the milk treated was 6–8 %.

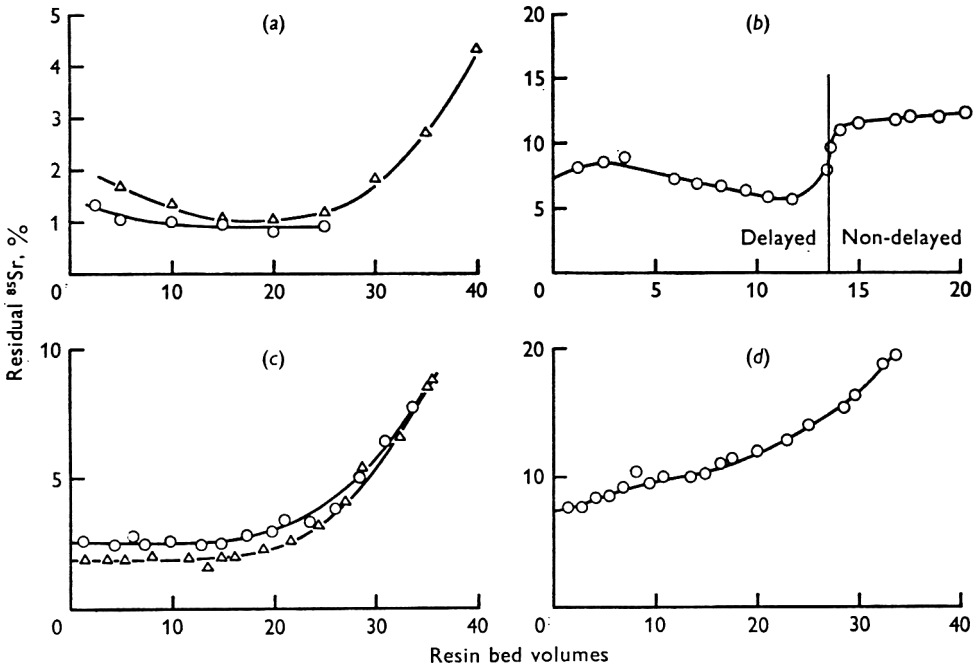

Fig. 1. Curves of concentration of residual ^{85}Sr against volume of milk treated. (a) On a laboratory column. ^{85}Sr introduced *in vitro*. Milk acidified with citric acid. Triangles and curves define separate experiments. (*b*) On the pilot plant with and without a delay tank. ^{85}Sr introduced *in vitro*. Milk acidified with citric acid. (*c*) On the pilot plant. ^{85}Sr introduced *in vivo*. Milk acidified with citric acid. Triangles and curves define separate experiments. (*d*) On the pilot plant. ^{85}Sr introduced *in vitro*. Milk acidified with HCl.

This level of residual ^{85}Sr was considerably higher than had been obtained with a laboratory column but it was not known at the time that the pH electrode was giving a low reading (see Glascock *et al.* 1968) and that the milk had been passing through the column at a pH of about 5·5 instead of 5·3 as indicated on the meter. The efficiency of removal of radiostrontium by the resin is known to be very sensitive to pH (Edmondson *et al.* 1963). Our aim in this work was to remove at least 90 % of radiostrontium from milk. Since the residual concentration was expected to be as much as 4 % higher when the radiostrontium was introduced *in vivo* than when it

was introduced *in vitro* it appeared that 30 r.b.v. of milk was the maximum amount that could be treated. It was desired to treat at least 2300 l. of milk/day and the resin bed volume was therefore increased to 75 l.

Effect of delay between acidification and application of milk to resin

It was found by Easterly, Edmondson, Avants & Sadler (1964) that, with a delay of 1 h between acidification of the milk and its application to the resin, the efficiency of removal of radiostrontium increased from 89·9 to 93·5%. Experiments were therefore carried out on this plant for the purpose of confirming that observation. Milk containing ^{85}Sr introduced *in vitro* was kept acid for 1 h in the delay tank before application to the resin as previously described (Glascock *et al.* 1968). After the passage of 13 r.b.v. the delay tank was by-passed so that the milk was applied to the resin within about 4 min of acidification. The variation in concentration of residual ^{85}Sr with volume treated is shown in Fig. 1 (*b*). It will be seen that the concentration was doubled by the abolition of the holding period.

Table 1. *Residual ^{85}Sr, as percentage of the original concentration, in milk containing the nuclide introduced* in vitro *after the passage through the pilot plant of* 15, 20 *and* 30 *r.b.v. of milk acidified with citric acid*

	Residual ^{85}Sr, %		
R.b.v. treated ...	15	25	30
Expt 1	1·27	1·54	2·05
2	2·39	2·86	3·28
3	1·61	1·81	2·21
4	2·79	3·39	3·99
5	2·60	2·92	3·29
6	1·42	1·84	2·43
Mean	2·01	2·55	2·88

Expts 2–6 were those in which the effect of increasing ^{85}Sr concentration in regenerating solution was investigated. See text and Fig. 2.

This experiment also was carried out when the pH control was defective and the efficiency was therefore less than that obtained after it had been corrected. Nevertheless, the experiment demonstrated that the radiochemical efficiency was substantially improved by the use of the delay tank which was therefore retained as a permanent feature of the process.

When the defective pH electrode system had been replaced no further modifications of the plant appeared necessary and experiments were carried out to determine the efficiency of removal of ^{85}Sr introduced into the milk *in vitro*. Table 1 (expt 1) shows the residual activity in the total milk treated after the passage of 15, 25 and 30 r.b.v. through the plant. The total residual ^{85}Sr was just over 2% after the processing of 30 r.b.v.

The effect on radiochemical efficiency of re-use of part of the regenerating solution

The measurement of radiochemical efficiency by single tests on milk containing ^{85}Sr, even if it were introduced *in vivo*, might indicate a higher efficiency than would be obtained if the plant were in continual use for the removal of environmental

^{90}Sr. In these circumstances, three-quarters of the regenerating solution, having been used before (Glascock *et al.* 1968) would contain ^{90}Sr some of which would be left on the resin. Only the last quarter of the solution used would be fresh and if it were insufficient in quantity to displace all the residual ^{90}Sr on the resin the efficiency of removal of the nuclide from milk would be correspondingly reduced.

Five consecutive experiments with milk containing 0·2 $\mu c/l$. of ^{85}Sr introduced *in vitro* were therefore carried out and the resin regenerated after each experiment according to the usual programme. Table 2 shows the total residual ^{85}Sr found in the 4 tanks of regenerating solution *after* the regeneration of the resin which followed each experiment. The figures in parentheses show the number of times the

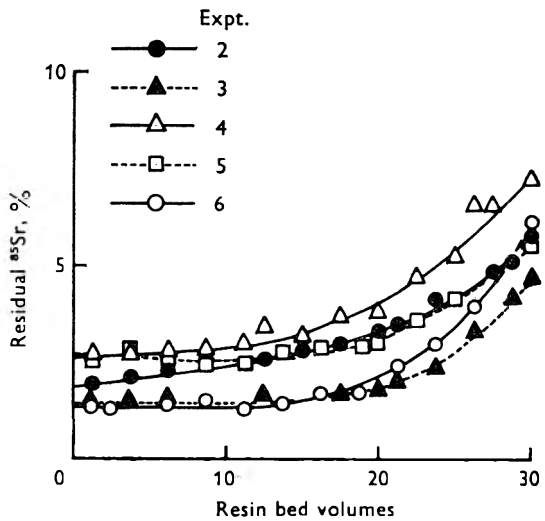

Fig. 2. Curves of concentration of residual ^{85}Sr against volume of milk treated. ^{85}Sr introduced *in vitro*. Milk acidified with citric acid. Five consecutive experiments to test effect of accumulation of ^{85}Sr in regenerating solution.

solutions in the tanks had been previously used. Only after expt 5 were 'steady state' conditions reached which were analogous to those which would be obtained if the plant were in continual use for the removal of environmental ^{90}Sr. The table also shows the total ^{85}Sr removed with nitric acid solution during the course of the cleaning which preceded regeneration. Negligible amounts of radioactivity were removed by the other washing procedures.

In the course of the experiments there was a small increase in the amount of ^{85}Sr removed by the solutions of tank 4 (which are thrown away after each experiment) and larger relative increases in the amount removed by the solutions in the other tanks. By the time expt 6 was carried out, however, the total amount of ^{85}Sr in the tanks was approaching a constant value and it is to be noted that the concentration in tank 1 at this point amounted to only 1·3 % of the total removed. This suggests that even smaller and therefore negligible amounts must have been left on the regenerated resin.

Figure 2 shows the variation of the concentration of residual ^{85}Sr with the volume of milk treated in these experiments. The cause of the small initial rise in the level

of residual activity followed by a fall in expt 5 is not known with certainty but it was probably due to small variations in pH.

Table 1 shows the total residual ^{85}Sr in all the milk treated after the passage of 15, 25 and 30 r.b.v. in these experiments and represents, of course, the integrals of the curves up to this point. In expt 2, as in expt 1 previously referred to, the resin had been treated with regenerating solution containing no ^{85}Sr, whereas that used in expts 3–6 contained increasing amounts as shown in Table 2. There was no correlation between the concentration of residual ^{85}Sr and the position of the experiment in the series. As was to be expected from the results in Table 2 the residual ^{85}Sr in part of the regenerating solution did not impair the total efficiency of removal from milk.

Table 2. *Total ^{85}Sr in the regenerating solution and HNO_3 (used for cleaning the resin bed) during the course of 5 experiments on the pilot plant, each with milk containing a total of* 453 μc ^{85}Sr *introduced* in vitro

(Numbers in parentheses indicate the number of times the solution in the tank had been used *before* the experiment.)

	Total ^{85}Sr, μc				
	Tank				HNO_3
Expt	4	3	2	1	
2	337 (0)	48·7 (0)	7·9 (0)	1·64 (0)	2·66
3	356 (1)	64·2 (1)	12·8 (1)	2·14 (0)	2·56
4	356 (2)	72·0 (2)	16·6 (1)	4·10 (0)	2·44
5	363 (3)	78·0 (2)	20·9 (1)	4·10 (0)	2·54
6	358 (3)	82·2 (2)	23·0 (1)	4·28 (0)	2·41

Experiments with milk containing ^{85}Sr introduced in vivo

Two separate experiments were carried out on the plant with 2300 l. of milk containing 0·210 and 0·176 μc/l. of ^{85}Sr introduced *in vivo*. Figure 1 (*c*) shows the variation in concentration of residual ^{85}Sr with volume of milk treated, and Table 3 shows the total amount removed after the passage of 15, 25 and 30 r.b.v. Comparison of these values with those obtained for milk containing the ^{85}Sr introduced *in vitro* (Table 1) shows that the efficiency of removal of the nuclide was not appreciably different whether it was introduced *in vivo* or *in vitro*.

Table 3. *Residual ^{85}Sr, as percentage of the original concentration, in milk containing the nuclide introduced* in vivo, *after the passage through the pilot plant of* 15, 25 *and* 30 *r.b.v. of milk acidified with citric acid*

	Residual ^{85}Sr, %		
R.b.v. treated ...	15	25	30
Expt 7	2·46	2·71	2·98
8	1·90	2·11	2·41
Mean	2·18	2·41	2·70

Effect of pH on efficiency of removal of radiostrontium by pilot plant

The effect of pH was not investigated systematically since it had already been done by Edmondson *et al.* (1963). Two experiments, however, were carried out on milk containing ^{85}Sr introduced *in vitro*, one at pH 6·8 and the other at pH 4·9.

At pH 6·8, the concentration of residual ^{85}Sr varied very little with the volume treated, increasing linearly from 46 % at the beginning to 52 % after the passage of 30 r.b.v.

The milk did not clot at pH 4·9 provided it was kept cold and the whole of the batch was successfully treated at a temperature which did not rise above 8 °C. In the first 15 r.b.v. treated, the concentration of residual ^{85}Sr was only 0·15 % but, as had been found in experiments at pH 5·2–5·3, it then began to increase until by the time 30 r.b.v. had been treated the concentration in the effluent milk was 4·75 %. The concentration in the whole 30 r.b.v. treated was then only 0·87 % which is less than half the value obtained at pH 5·25. This experiment is chiefly of interest in showing that milk can be treated at a pH below the usually accepted clotting point.

Use of hydrochloric acid for acidification

In all the experiments so far carried out on the plant, citric acid had been used to lower the pH as specified by Edmondson *et al.* (1962). For the special requirements of nutritional experiments it was decided also to prepare milk treated with hydrochloric acid during processing. A determination of radiochemical efficiency was therefore made when this acid was used. As found by Easterly *et al.* (1964) the efficiency of removal of ^{85}Sr introduced *in vitro* was then considerably less (Fig. 1 (*d*)) than with citric acid. The concentration of residual ^{85}Sr in the first 30 r.b.v. treated was 11 %.

Removal of ^{137}Cs with a laboratory column

Experiments with a laboratory column were carried out on milks containing ^{137}Cs introduced both *in vitro* and *in vivo* and acidified with citric acid to pH 5·25. Fig. 3 (*a*) shows the variation in concentration of residual ^{137}Cs with the volume treated.

Over the first 7 r.b.v. the process removed ^{137}Cs much more efficiently than ^{85}Sr. The breakthrough point occurred earlier, however, and the concentration of residual nuclide then rose more rapidly to much higher values. Although the curves are not quite superposable, the concentration of residual ^{137}Cs in all the milk treated up to 30 r.b.v. was 22·7 % in both experiments showing, as expected, that the nuclide is removed with the same efficiency whether introduced into the milk *in vivo* or *in vitro*. Experiments on the pilot plant were therefore carried out only on milk containing the nuclide introduced *in vitro*.

The removal of ^{137}Cs with pilot plant

Two experiments were carried out on milk containing ^{137}Cs introduced *in vitro* one after acidification with citric acid to pH 5·25 and one at pH 6·8 (Fig. 3 (*b*)). As had been found on a laboratory column the breakthrough of ^{137}Cs in acidified milk

occurred earlier (10 r.b.v.) than that of ^{85}Sr. In neutral milk however, the breakthrough occurred later (15 r.b.v.) and the concentration in the 30th r.b.v., although much greater than that ever reached by ^{85}Sr was considerably less than that in acidified milk. Consequently, the concentration of residual ^{137}Cs in all milk treated up to that point was only half as great in neutral as in acidified milk (Table 4). Edmondson (1964) also reported more efficient removal of ^{137}Cs from neutral than from acidified milk.

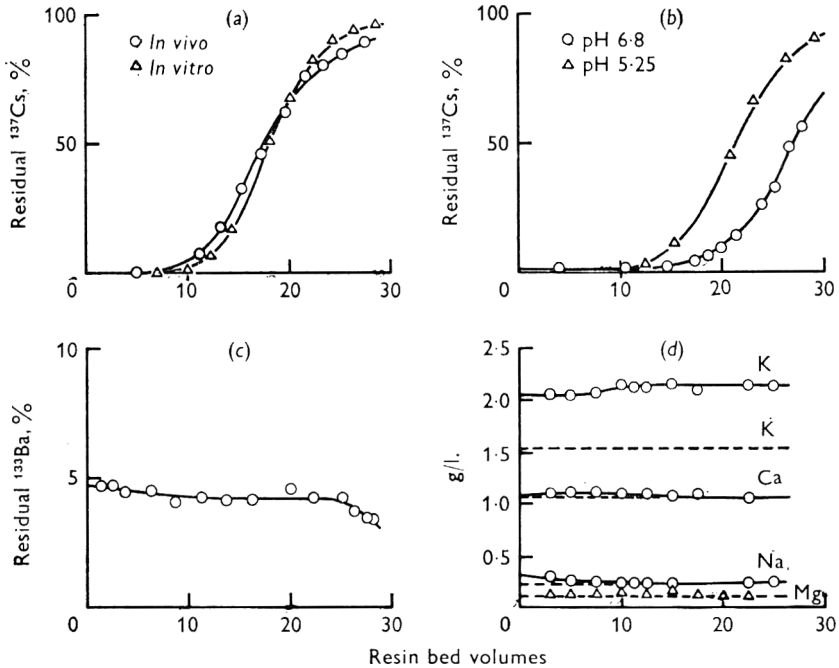

Fig. 3. (*a*) Curves of concentration of residual ^{137}Cs against the volume treated in a laboratory column. ^{137}Cs introduced *in vivo* and *in vitro*. Milk acidified with citric acid. (*b*) Curves of concentration of residual ^{137}Cs against the volume treated in a pilot plant. ^{137}Cs introduced *in vitro*. Milk at pH 6·8 and acidified with citric acid to pH 5·25. (*c*) Curve of concentration of residual ^{133}Ba against the volume treated in a pilot plant. ^{133}Ba introduced *in vitro*. Milk acidified with citric acid. (*d*) Curves of concentration of K, Ca, Na and Mg against the volume of milk treated in a pilot plant. Dotted line: normal concentration of element. Solid line: concentration found.

Removal of radiobarium with pilot plant

Since ^{140}Ba (half-life 12·8 days) is also a constituent of fall-out, an experiment was carried out on the plant to determine its efficiency for the removal of this element. For experimental purposes it was found more convenient to use ^{133}Ba (half-life 7·5 years) than ^{140}Ba. The nuclide was introduced into the milk *in vitro*.

Figure 3 (*c*) shows the variation with the volume treated in the concentration of ^{133}Ba in the effluent milk. It remained between 4 and 5% over the whole of the experiment with a slight decline towards the 25th r.b.v. Although environmental radiobarium is of little danger to health owing to its short half-life, this experiment shows that it is removed from 30 r.b.v. by the pilot plant with about the same efficiency as radiostrontium.

ANALYTICAL RESULTS

Acid and alkali additions

The solutions used on the plant for acidification and neutralization were 2·5N citric and hydrochloric acids and potassium hydroxide. The amount of citric acid required to reduce the pH of the milk to 5·25 was found to be 1·73 ml/100 ml of milk, but the amount of potassium hydroxide required to restore the pH to 6·8 was only 75 % of that calculated. Both these values were confirmed by acidification of the milk in the laboratory and back-titration. No measurements of the amount of hydrochloric acid used on the plant were made but laboratory experiments showed that a somewhat greater volume (1·88 ml/100 ml of milk) of 2·5N hydrochloric acid than of citric acid was required. The potassium hydroxide required to restore the milk to neutral, however, was 92 % of theoretical. From these data the total additions to the milk have been calculated and are shown in Table 5. The total amounts of water and salt added were about 3 and 0·3 % of the milk, respectively.

Table 4. *Residual ^{137}Cs, as percentage of the original concentration, after the passage of* 15, 25 *and* 30 *r.b.v., with and without acidification with citric acid during processing in the pilot plant*

	Residual ^{137}Cs, %		
R.b.v. ...	15	25	30
pH 5·25	1·2	16·3	27·5
pH 6·8	1·73	5·95	13·6

Table 5. *Additions to milk during processing*

	Acid used for acidification	
	Citric	Hydrochloric
2·5 N-acid added/100 ml milk, ml	1·73	1·88
2·5 N-KOH added/100 ml milk, ml	1·29	1·73
Citric acid or chloride added, mg/100 ml	277	167
K added, mg/100 ml	126	169
Salt formed, based on K used, mg/100 ml	338	323
Water added, ml/100 ml milk		
(*a*) with solutions	3·02	3·61
(*b*) water of neutralization	0·07	0·08
Total	3·09	3·69

Table 6 shows the concentration of the principal constituents, the freezing-point depression and enzymic clotting time of untreated milk, of milk treated with citric acid and of milk treated with hydrochloric acid during processing. For brevity, these 2 kinds of treated milk are referred to as citric acid-treated milk and hydrochloric acid-treated milk in the following paragraphs.

Organic constituents

The small but statistically significant differences between the concentrations of nitrogen, fat and SNF become even smaller when allowance is made for the 3–4 % of water added to the milk with the acid and alkali during processing. There was

therefore no appreciable loss of the main nutritional ingredients of the milk during processing. Furthermore, gas chromatographic analysis has shown that the fatty acid composition of the milk fat was not changed by processing. These results are in agreement with those of Isaacks *et al.* (1967).

Table 6. *Analysis of milk*

(Means, standard error of means (S.E.M.) and significance tests.)

Observation	Control (X)	Treatment with HCl (H)	Treatment with citric acid (C)	S.E.M. (6 D.F.)	Results of significance tests *
Total nitrogen, mg/100 g	508	500	492	2·7	C H X
Fat, %	3·81	3·73	3·73	0·021†	C H X
SNF, %	8·46	8·42	8·54	0·031†	H X C
Freezing-point depression, °C	0·523	0·618	0·569	0·0013	X C H
Chloride, mg/100 g	97·5	189·2	98·2	0·40	X C H
Citric acid, mg/100 ml	214	209	413	8·2	H X C
K, mg/100 g	131	224	208	2·5	X C H
Na, mg/100 g	54·8	57·5	52·5	0·43	C X H
Mg, mg/100 g	11·9	11·6	12·2	0·88	H X C
Total Ca, mg/100 g	120	104	124	1·7	H X C
Soluble Ca, mg/100 ml	40·2	42·8	53·5	0·51	X H C
Total P, mg/100 g	91·5	90·0	85·8	0·82	C H X
Soluble P, mg/100 ml	24·0	28·5	35·0	0·55	X H C
Enzymic clotting time	20 min	3 min	> 24 h	—	— — —

* Treatments underscored by the same line were not significantly different at P = 0·05.
† S.E.M. with 38 D.F.

Freezing-point depression

The mean freezing-point depression of all the milk samples and the concentration of SNF in untreated milk are lower than normal to a degree which implies the introduction of 3–4 % of water (additional to that added with acid and alkali) at some point before analysis. It seems possible that this occurred in the pasteurizing plant where batches of less than 200 l.. which were rather small for the size of the plant, were treated before being used for the nutrition experiments.

The extra potassium chloride found in the processed milk would be expected to produce a further depression of freezing point of 0·093 °C which is in good agreement with the observed difference in freezing point depression (0·095 °C). The effect of extra citrate on a complex system such as milk cannot be calculated but clearly it would cause some further depression of freezing point. The small amount of extra soluble calcium and phosphorus would also contribute to the increased depression especially in citric acid-treated milk (see Table 6).

Inorganic constituents and citrate

Figure 3 (*d*) shows the variation with volume treated of the 4 principal cations during the processing of 25 r.b.v. of milk acidified with citric acid. The concentration of the ions remained nearly constant during the whole course of the treatment. Table 6 further shows the small diminution in concentration of phosphate and

citric acid in hydrochloric acid-treated milk which was expected to result from the water added with acid and alkali. In citric acid-treated milk, however, no such difference in chloride content was observed and the concentration of total phosphorus was inexplicably low. Apart from potassium, citric acid and chloride, however, which were added during the process, none of the constituents was outside the normal range.

The enzymic clotting time of hydrochloric acid-treated milk was less than normal which is in agreement with the observations of Odagiri & Nickerson (1965). They attributed this effect to an increase in soluble calcium which was also observed in our work (Table 6). The abolition of clotting in citrate-treated milk was presumably due to sequestration of the calcium by citrate ions (see below).

Table 6 shows that treatment with citric acid produced a considerable increase, and that hydrochloric acid produced a somewhat smaller increase, in soluble calcium and phosphorus. These results suggest that calcium liberated from protein or from colloidal calcium phosphate by acidification with citric acid is sequestered by citrate ions which hinders or prevents its complete return to its original state of combination after neutralization. This mechanism could also operate to some extent in hydrochloric acid-treated milk in which the concentration of naturally occurring citric acid is about half of that found in citric acid-treated milk.

Comparison of the figures in Table 6 with those in Table 5 reveals some interesting facts, some anomalous. Table 5 shows that when citric acid was used for acidification 277 mg/100 ml were added to the milk. The difference between the citric acid contents of the treated and untreated milks (Table 6), however, was only 199 mg/100 ml which indicates a net loss of 78 mg citrate/100 ml. If, as suggested by Easterly *et al.* (1964) citrate forms cationic complexes with alkaline earths some would be removed by the resin bed. That explanation, however, cannot be applied to the imbalance in chloride: when hydrochloric acid was used for acidification the total chloride added was 167 mg/100 ml (Table 5), whereas the difference in chloride content between the treated and untreated milks (Table 6) was only 91·7 mg/100 ml.

The difference in potassium content between untreated milk and milk acidified with citric and hydrochloric acids was 77 and 93 mg, respectively. This is 61 and 55 % of the potassium known to be added and implies a net loss. It is difficult to understand how this could have occurred on the resin bed as there was no corresponding increase in concentration of any of the other cations.

Toxic metals, iron and stable strontium

As data on the maximum concentrations of toxic metals were not available for all the chemicals used in the process an analysis was carried out on typical samples of untreated milk and of milk treated with citric acid during processing. These results, together with data on iron and stable strontium, are shown in Table 7.

The concentration of antimony was increased during treatment from a value of less than 0·05 ppm. to 0·24 ppm. There is no legal limit in Britain for the concentration of antimony in food, but according to Monier-Williams (1949) any concentration above 1 ppm. in liquid food is undesirable. The concentration of copper was also raised from a value of less than 0·1 ppm. to 0·4 ppm. This concentration is far too small to be toxic but could possibly favour the oxidation of fat and the production

of off-flavours in liquid or in dried milk if it were kept in store for long periods The small increase in the concentration of iron is of little importance.

Although large doses of stable strontium can cause rickets the concentration found in the treated milk was far too low to be objectionable. It is interesting to note that although the process removed nearly all the original labelled strontium, it was replaced by about 6 times as much of the unlabelled element. This stable strontium probably occurred as an impurity in one of the chemicals in the regenerating solution.

Table 7. *Toxic metals, iron and stable strontium in milk acidified with citric acid during treatment*

	Concentration, mg/l.	
Metal	Untreated milk	Treated milk
Lead	< 0·05	< 0·05
Copper	< 0·1	0·4
Arsenic	< 0·02	< 0·02
Antimony	< 0·05	0·24
Iron	1·00	2·00
*Strontium	0·32	1·96

* Analysis carried out on a different batch of milk from that of the other metals.

Table 8. *Effect of processing on vitamin content of milk acidified with citric acid*

	Concentration in milk, mg/l.	
Vitamin	Before treatment	After treatment
Riboflavine	2·0	1·9
Pantothenic acid	5·4	5·9
Vitamin B_{12}	0·0031	0·0032
Vitamin B_6	0·40	0·34
Biotin	0·023	0·022
Nicotinic acid	0·69	0·50
Folic acid	0·045	0·043
Thiamine	0·40	0·13
Vitamin A	0·35	0·34
Carotene	0·078	0·074
α-Tocopherol	0·79	0·79

Vitamins. Table 8 shows the vitamin content of milk before and after treatment in the plant when citric acid was used for acidification. Appreciable losses were observed in thiamine, vitamin B_6 and nicotinic acid whose concentrations were reduced to 33, 85 and 73 % of their original values. Although removal of vitamin B_6 was not observed by Isaacks *et al.* (1967) some loss during treatment was not unexpected as it is weakly basic and might therefore be exchanged for other cations on the resin. Thiamine is more strongly basic and the loss observed both in the present work and by Isaacks *et al.* was most probably due to exchange on the resin. The loss of 27 % of nicotinic acid is inexplicable.

Appearance and flavour

Treated milk had a deeper yellow colour than untreated milk, and it was noticed that this was produced by the addition of alkali at the neutralization stage whether or not the milk had been passed through the resin bed. On centrifugation, treated milk gave a more bulky sediment and a less turbid aqueous phase than untreated milk. Furthermore, the aqueous phase from citric acid-treated milk was distinctly less turbid than that from hydrochloric acid-treated milk. A difference in turbidity between treated and untreated milk was also observed when the cream was allowed to separate by gravity.

A triangular flavour test in citric acid-treated milk was made with the help of 22 members of the Institute staff, none of them specifically trained in flavour evaluation. In this test, each participant was offered 3 samples of milk of which 2 were identical and 1 was different (untreated or treated milk). They were asked to pair the identical samples and identify them. Fifteen correctly identified plant-treated milk. In addition to identifying the samples, the participants were asked to comment. The results of this test, together with the comments of the participants, are summarized in Table 9. It is clear that many of the criticisms were contradictory, and although a highly significant number ($P = 0{\cdot}0009$) of subjects correctly identified plant-treated milk, all but one agreed that it was acceptable in flavour and that they could not have identified it without immediate reference to untreated milk. The one (female) participant who dissented, actively disliked plant-treated milk.

Table 9. *Triangular flavour test on milk acidified during processing with citric acid*

Number of subjects	22
Number making correct choice	15
Probability of this result arising by chance	0·0009

Comments on processed milk	
Comment	No. of subjects
Sweeter	4
Less sweet	4
Harsh	2
Sour	2
Bitter	2
Metallic	1
More creamy	1
Less salty	1
Cooked	1
Cardboard	1

Preparation of roller- and spray-dried powder

If this process were ever operated on a national scale the product would probably be distributed in powder form. Batches of 250 l. of citric acid-treated milk were therefore subjected to both roller- and spray-drying in pilot plants belonging to a commercial firm. This milk was processed without difficulty in both plants and the

products were normal in all respects including flavour and appearance. Later, batches of 5500 l. of both citric acid- and hydrochloric acid-treated milk were successfully roller-dried also to yield normal products.

DISCUSSION

Radiochemical efficiency

Tables 1 and 3 show that after the processing of 30 r.b.v. of milk the mean residual concentration of ^{85}Sr was 2·88 % when the nuclide had been introduced *in vitro* and 2·70 % when it had been introduced *in vivo*. The expected difference of up to 4 % between the efficiencies of removal of ^{85}Sr introduced *in vitro* and *in vivo* was therefore not observed. The mean value for all the experiments was 2·83 % with a range of 2·05–3·99 %. This is very satisfactory and better than the arbitrary value of 10 % which was set as the object of this work. There was an interval of 6 months between expt 1 and expts 2–6 (Table 1) during which time 35 batches of 2300 l. of milk had been passed through each of the resin beds. There was no evidence that the radiochemical efficiency of the process decreases with repeated use of the resin.

Sadler *et al.* (1967) obtained a mean residual concentration of 5 % of environmental ^{90}Sr with their pilot plant, and Fooks, Terrill, Heinemann, Baldi & Walter (1967) one of 7·9 % with a large-scale plant. There is no reason to think that the difference in efficiencies between the Shinfield and American plants is connected with the strontium isotope used for measurement. The pH used by Sadler *et al.* (5·3 ± 0·05) and Fooks *et al.* (5·35) was marginally higher than that used in the Shinfield plant (5·25 ± 0·03). They might have obtained improved efficiency had they operated at a slightly lower pH but apparently they did not do so for fear of clotting the milk. The present work, however, shows that milk can be successfully treated at a pH as low as 4·9 provided it is kept cold. It is probable that the greatest contribution to the higher efficiency of the plant at Shinfield was made by the delay between acidification of the milk and its application to the resin.

The lower efficiency of removal of ^{85}Sr when hydrochloric acid was used instead of citric acid during processing is in agreement with the findings of Easterly *et al.* (1964) and if for any reason an increased concentration of citrate in the treated milk were considered undesirable the concentration of residual radiostrontium of 11 % would be just acceptable.

The breakthrough point of radiocaesium was earlier than that of radiostrontium as was shown by Edmondson (1964) who also found a less efficient removal of radiocaesium from acidified than from neutral milk. The reason for this is not known and was not in fact observed by the same author in earlier experiments (Murthy, Masurovsky, Campbell & Edmondson, 1961). The effect is of little practical importance, however, since environmental radiocaesium is always accompanied by radiostrontium which is removed with poor efficiency at neutral pH and which constitutes the greater danger to health. If 30 r.b.v. are treated, the concentration of radiostrontium is reduced to about 3 % but that of radiocaesium only to 30 %. On the other hand, if 15 r.b.v. are treated the concentration of both nuclides is reduced to 2 % or less. Maximum decontamination of milk could thus be achieved either by halving the day's throughput or by doubling the volume of the resin bed. The

design of a plant with 10 times the capacity of the pilot plant has been discussed in a preceding paper (Glascock *et al.* 1968) from which it will be seen that a further increase in volume of the resin bed might create technical difficulties.

Chemical composition

The processed milk showed no important changes in composition which were likely to affect its nutritional value. Total fat, nitrogen and SNF remained virtually unchanged and there was an appreciable deficiency of only one vitamin, thiamine, whose concentration could easily be restored by the addition of the vitamin to the milk.

The concentrations of citrate and chloride were increased in citric acid and hydrochloric acid-treated milks and the concentration of potassium in both. The small increase in the concentration of potassium chloride is unlikely to have any deleterious effect and the extra citrate is of importance only because it hinders enzymic clotting. However, if the process were ever applied on a national scale, the product would be primarily intended for infants and citrate is sometimes added to milk for feeding babies.

In order to keep the cost of the process as low as possible the cheapest available industrial grade chemicals were used for the regenerating solution. This could have been undesirable only if their use resulted in the introduction of toxic metals into the milk. As this did not occur there appears to be no reason why, subject to adequate analytical control, this grade of chemical should not be used.

We acknowledge with thanks the most valuable help of many of our colleagues in this work and in particular Dr J. E. Storry for the determination of citrate; Mr A. B. McAllan for the determination of magnesium; Mr A. W. Wagstaff for the determination of most of the other constituents of milk; Drs J. E. Ford and S. Y. Thompson for the determination of vitamins; and Miss Z. D. Hosking for statistical analysis. We thank Dr R. S. Bruce of the Agricultural Research Council Radiobiological Laboratory, Wantage, for the determination of stable strontium; Mr F. A. Lyne, Public Analyst, for the determination of toxic metals and iron; Dr R. J. MacWalter and Mr P. A. Hoare of Unigate Ltd. for organizing the drying of processed and unprocessed milk; the colleagues who took part in the flavour evaluation; and Mr G. A. Payne for technical assistance. We also thank Dr R. Scott Russell, Director of the Agricultural Research Council Radiobiological Laboratory, Wantage, and Drs S. J. Rowland, R. Aschaffenburg and J. W. G. Porter for most helpful conversations.

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The effect of intramammary infection during the dry period on the milk production of the affected quarter at the start of the succeeding lactation

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(*Received* 25 *January* 1968)

Summary. Intramammary infection present in quarters of dairy cows, either throughout the dry period or originating in the dry period, reduces the milk yield after calving. Relative to the milk yield of equivalent non-infected quarters of the same udder the reduction was found in this work to be about 35%. The relative milk yield of quarters found infected in late lactation was depressed by 48% but if the infection was eliminated during the dry period, the depression in milk yield was only 11% after parturition.

Quarters infected with staphylococci or streptococci normally yield less milk than do equivalent uninfected quarters and the milk produced has a lower solids content (Minett & Martin, 1936; White, Anderson, Johnson, Plastridge & Weirether, 1937; Crossman, Dodd, Lee & Neave, 1950; McLeod & Wilson, 1951; Watts, 1951; Rowland, Neave, Dodd & Oliver, 1959; O'Donovan, Dodd & Neave, 1960; Car, 1961; Wheelock, Rook, Neave & Dodd, 1966). When infection is eliminated from a quarter during lactation the milk yield relative to uninfected quarters within the same udder does not recover. However, at the start of the following lactation the yield shows a considerable, but not complete, recovery provided the quarter has remained uninfected during the non-lactating stage. This is in contrast to the changes in milk composition which usually shows a partial recovery as soon as the infection disappears, completely normal milk being obtained in the next lactation (Rowland *et al.* 1959; Wheelock *et al.* 1966).

The object of this study was to measure the effect, on the milk yield of a quarter after calving, of subclinical infections present at the end of the previous lactation and during the dry period: in particular to compare the effect of infections that persist throughout the dry period with the effect of those that are present at drying-off and disappear during the dry period, and also with the effect of those that start in the dry period and persist until calving.

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METHOD

This analysis has been made from the same experimental records as were used for the investigations of Crossman *et al.* (1950) and Rowland *et al.* (1959). The records were collected from a herd in which the cows were milked at regular intervals with a milking machine designed for the collection of milk from individual quarters. Regular bacteriological tests were made on milk samples taken from individual quarters of all the cows to determine the presence of udder infection. Full details of the method of sampling and diagnosis have been reported by Crossman *et al.* (1950). In general a quarter was diagnosed as infected either at drying-off or at calving if the same pathogen was recovered from 2 consecutive milk samples. Positive identification in a single sample was accepted as indicating infection only when clinical signs of mastitis were apparent. Quarters were always sampled after the last milking of lactation and on the day of calving, and a quarter uninfected at drying-off but infected at calving was accepted as having become infected in the dry period.

The measurements of the milk yields of the individual quarters were those taken before drying-off and the first after parturition for 105 cows. These were recorded in the last month of lactation and the first month after calving. On each of these occasions quarter yields were recorded at 4 consecutive milkings.

To measure the effect of infection in the dry period on the milk yield of a quarter the yield of the affected quarter has been expressed as a percentage of the yield of the adjacent non-infected quarter. This ratio is designated as the 'relative milk yield' in the tables. To measure the effect of changes in the infection state occurring during the dry period the relative milk yield at calving has been compared with the value before drying-off for the same quarters. As a control, the same estimates have been made on 210 pairs of quarters of cows that were found to be not infected either at drying-off or at calving. In these measurements the yield of the quarter on the left side of the udder was expressed as a percentage of that on the right side.

Results were available for 25 quarters infected at drying-off with the infection persisting throughout the dry period into the next lactation. Twenty-three of these infections were caused by staphylococci and 1 by streptococci. In addition, 19 quarters were found to be infected at drying-off, but not at calving. Thirteen of these infections were caused by staphylococci, 2 by streptococci and 4 by other pathogens. Records were also available of 36 quarters uninfected at drying-off, but which were infected at calving. Twenty of these infections were caused by staphylococci, 11 by streptococci and 5 by other pathogens.

RESULTS AND DISCUSSION

The results of the analyses are summarized in Tables 1 and 2.

The variation in milk yield between equivalent quarters of the same udder uninfected from drying-off to calving was small and similar to that reported by Johansson & Korkman (1952) (Table 1). This ratio was, however, considerably changed by udder infection present at some time between drying-off and calving. Quantitatively, the biggest depressions in yield occurred with infections present at calving, and the effects were approximately equal for infections that had persisted from drying-off

to calving and for those that had started in the dry period. Table 2 indicates that the relative yields of quarters infected throughout the dry period were on average similar in late lactation and after calving, though this would not be so if the infection became mastitic at calving. The results in Table 1 are a further demonstration that secretory tissue damaged by udder infection is not completely repaired during a dry period, even if the infection has been eliminated at drying-off. Nevertheless, the further analysis given in Table 2 shows that in this work the depression was only about 11 % at calving compared with 48 % at drying-off.

Table 1. *The effect of infection on milk yield at calving*

	No. of quarter pairs (n)	Relative yield*	Standard deviation	Yield depression, %
A. Quarters free from infection at drying-off and calving	210	100·7†	5·8	–
B. One quarter of a pair infected at drying-off but not at calving	19	88·7	16·9	11·3
C. One quarter of a pair infected at drying-off and at calving	25	67·8	15·1	33·2
D. One quarter of a pair becoming infected in the dry period	36	63·4	17·1	36·6

* Relative yield: in non-infected quarter pairs the yield of the left quarter has been expressed as a percentage of yield of the right quarter. In quarter pairs with one quarter infected at drying-off or at calving or at both, the yield of such a quarter has been expressed as a percentage of the yield of the non-infected quarter of the pair.

† Any mean values of 'relative yield' that are not bracketed together are significantly different at the 1 % level of significance, except that the difference between the 'relative yields' B and C is significant only at the 5 % level.

Table 2. *Changes in the relative milk yield in quarters freed of infection at drying-off and quarters remaining infected from drying-off to calving*

	No. of quarter pairs (n)	Relative yield*	Standard deviation
One quarter of a pair infected at drying-off but not at calving			
Yield at drying-off	19	52·0	35·9
Yield at calving	19	88·7	16·9
One quarter of a pair infected at drying-off and the infection persisting to calving			
Yield at drying-off	25	68·4	12·8
Yield at calving	25	67·8	15·1

*See Table 1 for definition.

The variation in the relative yields of quarters given in this paper do not indicate the true loss in milk production of the animals since there is the possibility that the secretory activity of the non-infected quarters may have increased at this time and

may have compensated, at least in part, for the damage caused by infection (Hansson, 1946; Turner, 1952).

These data are a further demonstration of the considerable physiological significance of the changes that occur in the mammary gland of the cow during the non-lactating stage between consecutive lactations. They also confirm the value of eliminating udder disease at drying-off by intramammary infusion of antibiotic (Smith *et al.* 1967).

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The use of single or composite milk samples for the determination of fat

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Summary. Samples were tested for fat percentage from every consignment of herd bulk milk from 10 herds over a period of 12 months. The mean monthly within-herd variance was 0·043. Also, triplicate samples were taken from 8 herd bulk supplies for 8 days and tested in duplicate and an analysis of variance was applied to the within-herd fat percentage in order to find the contribution sampling, testing and biological variances. It is shown that when dealing with tanker-collected milk where composite samples are not used the testing accuracy is secondary to frequency of sampling. By combining the variances in the formulae given, the accuracy of different systems may be obtained and compared using either single tested or composite samples.

INTRODUCTION

The so-called 'true' butterfat content of herd bulk milk supplies has been described as the simple average of the results of tests on fresh samples from each delivery over a month (Hermann & Anderson, 1965). It is common practice when dealing with can milk supplies to sample daily at the dairy to which the milk is delivered and to form a composite sample which is then tested after periods such as 7, 10, 14 days or 1 month. However, with the advent of bulk-collected milk this system has not been possible as the sample must be taken at the farm and transportation of samples, and bottles containing part composite samples, every day on a tanker gives rise to churning. This may be overcome by transferring single samples uplifted daily at the farms to a composite sample bottle at the factory or tank haulier's premises. This, however, gives rise to extra work, as fresh sample bottles are required every day and extra time is required for transferring the fresh sample to the composite sample bottle.

The purpose of the present study was to consider the variability of the butterfat content in herd milk supplies arising from day-to-day biological variance, sampling and testing, and to consider ways of assessing the butterfat content of tanker-collected milk without the use of composite samples.

The effect of testing accuracy on the monthly assessment was also considered.

METHODS

Samples of herd bulk milk were taken daily for a period of 12 months from 10 herds of from 28 to 74 cows. The milks had been stored in bulk tanks which conformed

to B.S. 696, (British Standards Institution, 1966). The milk had been mechanically agitated for a minimum period of 2 min prior to sampling. These samples were tested for butterfat by the Gerber method (British Standards Institution, 1955), using calibrated glassware and reagents conforming with the standard. In addition, triplicate samples were taken in the same manner from 8 herd bulk supplies for a period of 8 days. These samples were tested in duplicate by the use of the Milko-Tester Mark II (Brems, 1965).

RESULTS AND DISCUSSION

Table 1 gives the monthly and yearly variance for the 10 herds for which samples were tested from every consignment. The monthly variance for butterfat ranged from 0·0016 to 0·1086. The greatest change in fat content from one day to the next was 1·00% in a single herd. The mean yearly fat variance was 0·043. The mean monthly variance was 0·025.

Table 1. *The 'between-day variance' in milk fat percentage for individual herds, sampled daily for* 12 *months in* 1965–6

Month	1	2	3	4	5	6	7	8	9	10	Mean
April	0·0127	0·0165	0·0131	0·0089	0·0432	0·0604	0·0720	0·0103	0·0191	0·0369	0·0293
May	0·0291	0·0370	0·0201	0·0465	0·0584	0·0282	0·0818	0·0271	0·0213	0·1086	0·0458
June	0·0637	0·0122	0·0134	0·0355	0·0257	0·0206	0·0123	0·0169	0·0138	0·0260	0·0240
July	0·0203	0·0079	0·0540	0·0344	0·0216	0·0390	0·0217	0·0244	0·0343	0·0296	0·0287
Aug.	0·0184	0·0246	0·0206	0·0438	0·0511	0·0320	0·0640	0·0170	0·0266	0·0292	0·0327
Sept.	0·0236	0·0170	0·0067	0·0261	0·0186	0·0534	0·0456	0·0417	0·0288	0·0289	0·0290
Oct.	0·0436	0·0317	0·0292	0·0278	0·0236	0·0586	0·0636	0·0470	0·0479	0·0085	0·0381
Nov.	0·0117	0·0104	0·0135	0·0159	0·0149	0·0126	0·0759	0·0181	0·0098	0·0220	0·0205
Dec.	0·0051	0·0040	0·0080	0·0637	0·0129	0·0036	0·0132	0·0126	0·0080	0·0116	0·0143
Jan.	0·0175	0·0016	0·0016	0·0084	0·0091	0·0105	0·0119	0·0065	0·0089	0·0088	0·0085
Feb.	0·0066	0·0240	0·0240	0·0062	0·0107	0·0063	0·0070	0·0069	0·0107	0·0268	0·0129
Mar.	0·0113	0·0020	0·0020	0·0087	0·0367	0·0154	0·0059	0·0390	0·0036	0·0187	0·0143
Mean	0·0220	0·0157	0·0172	0·0272	0·0272	0·0284	0·0396	0·0223	0·0194	0·0296	—
12-month variance	0·0502	0·0251	0·0302	0·0473	0·0471	0·0623	0·0641	0·0212	0·0392	0·0474	—

Mean monthly variance = 0·0246; mean yearly fat variance = 0·043.

Table 2. *Analysis of variance of the fat percentage of bulk milks in a balanced* 8 × 8 *two-factor experiment*

Cause	Sum of squares	Degrees of freedom	Mean square	*F*-test	Expected mean square
Days	8·5719	7	0·5103	111·5***	$\sigma_T^2 + 2\sigma_S^2 + 48\sigma_D^2$
Herds	22·0389	7	3·1484	21·71***	$\sigma_T^2 + \sigma_S^2 + \sigma_{D(H)}^2 + 48[\sigma_H^2]$
Days/herds interaction	7·1061	49	0·1450	31·68***	$\sigma_T^2 + 2_S\sigma^2 + 6\sigma_{D(H)}^2$
Samples	0·5859	128	0·004577	4·44***	$\sigma_T^2 + 2\sigma_S^2$
Residual	0·1980	192	0·001031	—	σ_T^2
	33·5008	383	—	—	—

*** Highly significant: $P < 0·001$

It follows, therefore, that the fat variances due to the different sources were:
Days $\sigma_D^2 = 0·0105$. Days/herds $\sigma_{D(H)}^2 = 0·0234$. Sampling $\sigma_S^2 = 0·0017$. Testing $\sigma_T^2 = 0·0010$.

To find the contribution of sampling and testing to the total within-herd fat variance an analysis of variance was carried out on a balanced 8×8 two factor (herds and days) experiment with replicate sampling and testing. This analysis of variance is given in Table 2. All variances were highly significant ($P = 0{\cdot}001$). It can be seen from Table 2 that the greatest variance is associated with the days/herd interaction.

As in previous work with total solids percentage (O'Keeffe, 1967*a*) the formula for combining the variance due to the different causes can be applied; hence, the variance of the mean fat content is given by

$$V_H = \frac{1}{d}\left(\sigma_D^2 + \sigma_{D(H)}^2 + \frac{\sigma_S^2}{s} + \frac{\sigma^2}{st}\right), \tag{1}$$

where d is the number of days, s the number of samples and t the number of tests within samples, i.e. testing each single sample taken.

Where composite samples are used and the samples are tested once only during the assessment period, formula 1 becomes

$$V_H = \left(\frac{\sigma_D^2 + \sigma_{D(H)}^2}{d} + \frac{\sigma_S^2}{ds} + \sigma^2\right). \tag{2}$$

For example, if the period of assessment were 1 month and biological variance ($\sigma_D^2 + \sigma_{D\,(H)}^2$) is 0·025 and the sampling and testing variance 0·0017 and 0·0010, respectively, then for single testing of 8 samples the variance of the mean V_H (using formula 1);

$$= \tfrac{1}{8}\,(0{\cdot}025 + 0{\cdot}0017 + 0{\cdot}001)$$
$$= 0{\cdot}0035.$$

However, if 30 samples/month are taken and tested, the variance of the mean V_H (using formula 1):

$$= \tfrac{1}{30}\,(0{\cdot}025 + 0{\cdot}0017 + 0{\cdot}001)$$
$$= 0{\cdot}0009$$

When a 30-day composite sample is tested, the variance of the mean V_H is obtained using formula 2:

$$= \tfrac{1}{30}\,(0{\cdot}025 + 0{\cdot}0017) + 0{\cdot}001$$
$$= 0{\cdot}0019$$

It must be emphasized that the testing variance of 0·0010 found during this 8×8 two factor experiment was very small, being equivalent to a standard deviation of 0·03. In comparison to this, under normal practical testing conditions, the standard deviation of the Milko-Tester Mark II has been found to be as high as 0·083 with an equivalent variance of 0·0069 (O'Keeffe, 1967*b*). For composite samples which are tested once only this could have a serious effect but it would be considerably lessened with frequent sampling, and testing of single samples as would be more appropriate for tanker samples. In the 3 examples given where the variances are 0·0035, 0·0009 and 0·0019, these variances would be 0·0042, 0·0011 and 0·0078, respectively, if the figure of 0·0069 is taken as the testing variance.

In the 12 months experiment the mean yearly fat variance was 0·043. This figure included contributions from sampling and testing in addition to biological variance. However, as the samples were tested singly the contribution due to sampling and

testing would be negligible. Similarly, the contribution due to sampling and testing in the case of the mean monthly variance of 0·025 would be very small.

It can be seen, therefore, from the examples given that frequency of sampling is the most important factor in the assessment of the fat percentage of herd bulk milk supplies and that testing accuracy is of secondary importance. A desired accuracy (i.e. any desired variance of the mean) may be obtained by the use of different values for the symbols in formula 1. Moreover, the greater the value of d (i.e. the frequency of sampling) in formula 1, the greater the degree of accuracy. Similarly, using this formula, the variances of the mean for individual cows in herd proving schemes may be determined.

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Electronic counting of cells in milk: examination of a chemical treatment for dispersal of milk fat

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SUMMARY. The chemical method of Tolle, Zeidler & Heeschen, (1966) for dispersing the fat in milk to allow electronic counting of the cells in suspension has been examined. The optimum conditions prescribed for obtaining the best estimates of cell counts were largely confirmed though inaccurate counts occasionally occurred. The reproducibility of counts made on the same milk samples was acceptable. For milk samples from individual quarters, cell counts determined after the chemical treatment and those after centrifugal separation of the fat could be related by a linear regression line of unit slope through the origin. The surface active liquid Lissapol NXP (I.C.I. Ltd.) appeared to be a suitable substitute for the Witopal CO (Chemischen Werken in Witten/Ruhr), an alkylphenol polyglycol ether, used by Tolle *et al.* (1966).

INTRODUCTION

Direct counting of the cells alone in whole milk with a Coulter counter is prevented by the presence of the fat particles whose size distribution largely coincides with that of the cells. However, the particles of fat may be removed by centrifuging the milk diluted with saline (Cullen, 1965, 1967; Phipps & Newbould, 1965, 1966; Read, Reyes, Bradshaw & Peeler, 1967) or dispersed by chemical treatment (Tolle *et al.* 1966). The chemical method requires the milk sample to be treated with formalin (1/500) for 24 h beforehand which renders the cells resistant, in the main, to the subsequent action on the milk of a hot mixture of a surface active agent, Witopal CO, and alcohol in saline which dissolves the fat; the cells may be counted after cooling the mixture. Recent tests (Dijkman, Schipper & Walstra, 1966) have shown the chemical method to be promising for testing large numbers of samples.

The present paper gives the results of a laboratory study of the chemical method in which the various steps in the procedure have been examined. A comparison has also been made of the cell counts obtained following the chemical treatment with those determined by the method previously described by Phipps & Newbould (1966) employing centrifugal fat separation. In addition, some other surfactants more common in the United Kingdom than Witopal CO have been tested.

METHODS AND MATERIALS

The counter. A model A Coulter counter and a tube with a 70 μm diam. orifice were used, the volume of suspension metered through the orifice for each count being 72 μl. The instrument was calibrated with latex particles of narrow size-range whose mean diameter was determined by direct measurements from electron micrographs and also by measuring particle arrays with an optical microscope (Kubitschek, 1961); both methods gave a diam. of 2·65 μm. Instrument calibration constants were determined for the electrolyte specified in the chemical method and for 0·9% saline and were used appropriately in the experiments that followed. Adjustments to the gain trim control were made when necessary to compensate for any deviation (1–3 °C) in sample temperature from that used in calibrations. Corrections to data for electrolyte background counts were made where necessary. The particle count at any chosen size threshold was taken to be the average of duplicate determinations. The customary corrections for coincidence losses were applied but such errors were minimized by diluting samples where necessary to keep corrections to less than 5% of the counts.

Cell recovery by the centrifuging technique. The technique used was as described previously (Phipps & Newbould, 1966) though some details of the apparatus were different. The centrifuge in the present experiments was a non-refrigerated machine (Baragyro, Baird and Tatlock Ltd., London) with a head taking 4 swing-out sample buckets. Centrifuging conditions equivalent to those previously used were obtained by choosing a speed-setting of the 6-stop control to give 3400 g and allowing 12 min for spinning. Sample temperatures during centrifuging varied from 20 to 30 °C. For gentle re-dispersion of sedimented cells, sample tubes were rotated at 48 rev/min on the inclined shaft of a small electric motor. Final vigorous mixing for 1½–2 min was done on a test-tube mixer (Whirlimixer, Fisons Scientific Apparatus Ltd., Loughborough). The suspending medium used was a filtered sterile solution of sodium chloride B.P. 0·9% (w/v) (Boots Pure Drug Co. Ltd.). A double syringe device (Di-Zippette, Jencons Ltd., Hemel Hempstead) was used to sample and dispense the milk and saline.

The equation correlating microscope and electronic counts was previously determined using a 50 μl metered volume (Phipps & Newbould, 1966). Since 72 μl was metered in the present instrument, the electronic count became $0{\cdot}139N \times 10^3$/ml and the equation giving estimated microscope counts, M, was therefore:

$$M = 1{\cdot}617 \times 0{\cdot}139N \times 10^3/\text{ml of milk},$$

where the factor 1·617 is the slope of the correlation graph found previously and N is the difference between the counts at the 7·5 and 15 μm thresholds. Corrections for coincidence losses were unnecessary since they were allowed for in the correlation factor. Cell counts obtained with the electronic counter and directly with the microscope (Newbould and Phipps, 1967) confirmed this relation within the 95% confidence limits found before.

Owing to the possible occurrence in milk of body cells other than leucocytes, the propriety of the term 'leucocyte count' as applied to cell counts in milk is questionable. In this paper 'cell counts' will refer to counts of somatic cells generally.

Cell recovery by chemical treatment of the milk. The chemical procedure followed has

been described by Tolle *et al.* (1966). The basic electrolyte was 0·9 % NaCl containing formalin (1/1000). The solution to dissolve the fat in the milk was prepared by heating to 55–60 °C a quantity of the electrolyte containing 2 % Witopal CO and 12 % filtered ethanol. A milk sample, previously treated with formalin (1/500) and stored at laboratory temperature for 24 h, was diluted 1/50 (by pipette) with this solution and heated for 15 min at 80 °C in a water bath. Cell counts were made with the electronic counter after the sample had been cooled.

RESULTS AND DISCUSSION

Dissolving the fat: heating time and surface active agent. The minimum time of 15 min at 80 °C recommended by Tolle *et al.* (1966) for dissolution of the fat with Witopal CO was confirmed. After heating for this time the count at room temperature had fallen to a steady value and remained constant for up to 30 min heating; thereafter the count tended to decrease. In these experiments the size-threshold dial of the counter was adjusted so that all particles greater than 5 μm apparent diam. were counted. Unaccountably, the fat in a few (2 or 3 %) of the milks tested did not appear to dissolve completely (cf. Cullen, 1967) in spite of prolonged heating; globules of fat, or perhaps a mixture of fat and surface active agent, could be seen in suspension in the treated milk. The counts on these particular milks were, therefore, falsely high because these unidentified globules were also being counted.

Small variations in the percentages of surface active liquid and ethanol appeared not to affect particle counts.

Several surface active compounds likely to have the fat-dissolving property of Witopal CO were tried. Teepol (Shell Chemicals Ltd.), Lubrol L (I.C.I. Ltd.), Pogol 600 (Croda Ltd., Goole) and Igepal CA-630 (of U.S.A. origin) were found to be unsuitable. Occasional success was obtained with Lissapol NX (I.C.I. Ltd.) but since this reagent tended to precipitate out of solution upon heating and cooling it could not be used. Lissapol NXP, on the other hand, showed this effect much less and as will be shown later proved to be as effective as Witopal CO. A heating time at 80 °C of 10 min was found suitable.

Variation of counts after dissolution of fat. Cell counts on milk samples treated with formalin for 24 h, then with the hot chemical solvent and cooled to laboratory temperature, remained constant for up to 2–3 h. Thereafter, a steadily increasing count was obtained, possibly because of swelling of small cells or because of a tendency for them to aggregate. After 4–5 h the number of particles counted decreased, possibly due to disintegration of aggregates and individual particles.

Size and count analyses in the present work were done within 1–2 h after cooling and were thus unaffected by these particular ageing effects.

The formalin treatment time. The dependence of cell size distributions on the formalin treatment time was studied. Milk samples were treated with formalin at room temperature at a dilution of 1/500 for periods of between 2 and 264 h. They were then subjected to the chemical treatment and cooled to room temperature before determining size distributions.

Cell size distributions, after formalin treatments lasting for 2, 7 and 24 h, of a milk containing about 15×10^6 cells/ml are given in Fig. 1. Changes in the positions

of the peaks and the troughs (at about 4 μm diam.) of these distributions are apparent. In Fig. 2 the peak and trough positions of the distributions for another milk (about $1{\cdot}3 \times 10^6$ cells/ml) have been plotted against duration of the formalin treatment. These results indicate that a relatively stable, reproducible distribution was obtained only after 24 h of treatment.

The increasing resistance of cells to the chemical treatment was indicated by the rapid increase in the cell count, taken at a size-threshold setting of the counter of

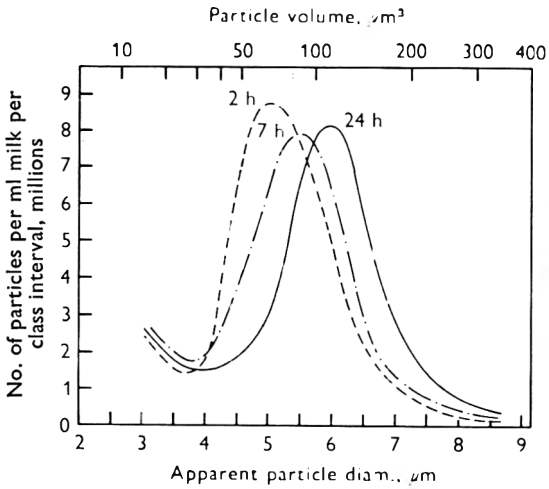

Fig. 1

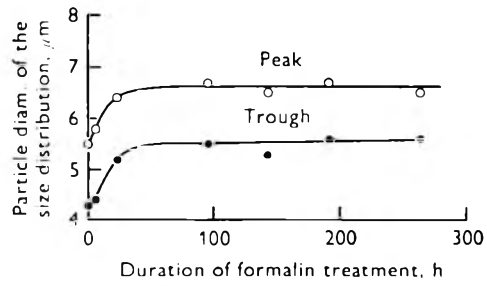

Fig. 2

Fig. 1. Particle size distributions in milk 2, 7 and 24 h after addition of formalin (1/500). Chemical method of fat dispersal. Cell content of milk: $1{\cdot}5 \times 10^6$/ml.

Fig. 2. Time variations of the peak and trough positions of the particle size distribution in a milk after treatment with formalin (1/500). Chemical method of fat dispersal. Cell content of milk: $1{\cdot}3 \times 10^6$ml.

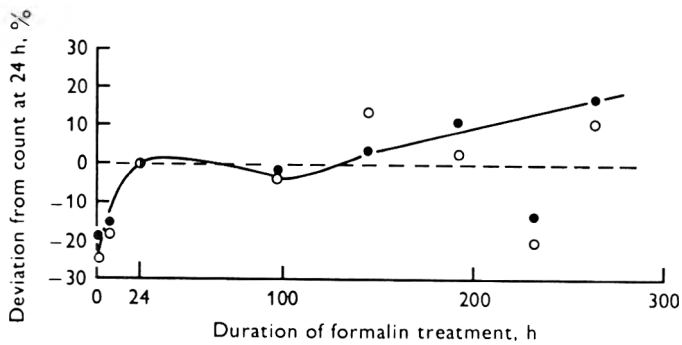

Fig. 3. Percentage variation of counts with time after addition of formalin to milk. Chemical method of fat dispersal. Milk ○, $1{\cdot}5 \times 10^6$ cells/ml; milk ●, $1{\cdot}3 \times 10^6$ cells/ml.

5 μm, as the formalin treatment time increased to 24 h. Figure 3 shows this effect and also indicates that 24 h (or possibly slightly less) would be the minimum time and about 4 days the maximum time permissible for formalin action. Other data obtained supported these results. The conclusions are largely in agreement with those of Tolle *et al.* (1966).

Comparison of particle size distribution curves. Using the Coulter counter, particle size distributions were determined in several milks of different cell contents which had received both chemical and centrifugal treatments. Some typical results are shown in Fig. 4. Chemical treatment resulted in variable amounts of extraneous material, composed possibly of coagulated protein, broken cells and even some fat globules, which affected the trough positions and masked the true minima in the cell distributions (cf. Dijkman *et al.* 1966). Peaks and troughs, when well-defined, always occurred at smaller diameters than those obtained following centrifugal separation. Experiments with cells dispersed in separated milk showed that both the formalin treatment and Witopal CO/heat process reduced the cell sizes.

With some milks, especially those of lower cell content, both peaks and troughs were absent.

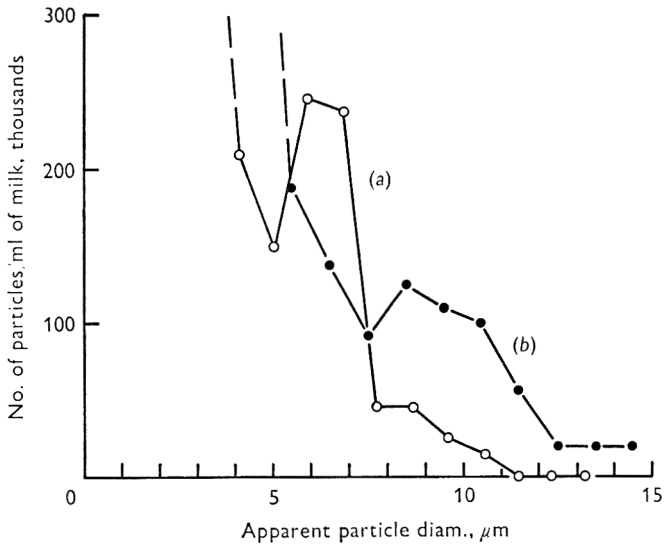

Fig. 4. Comparison of particle size distributions in a milk after (*a*) chemical, and (*b*) centrifuging treatment for fat.

Correlation of the cell counts obtained by the chemical and centrifuging methods. Variations in the particle distributions of different milks and the failure of the chemical method to separate the distribution of cells completely from other particulate matter, leads to difficulties in deciding the optimum threshold size setting of the counter for the best agreement of electronic and microscope counts. Tolle *et al.* (1966) did not state the precise threshold setting they used but calculations using the data they gave suggest that it was 4·5–5 μm. Dijkman *et al.* (1966) tested quarter, cow and herd samples and found that a 5 μm (65·5 μm^3) threshold setting gave good graphical agreement with microscope counts although the counts for milks of low cell content ($< 10^5$/ml) tended to be overestimated. Accurate counting in the range 10^5 to 10^6 cells/ml seems likely to be required for control of milk quality (British Veterinary Association, 1965).

Milk samples from individual quarters of cows were used in the present work. In Fig. 5, cell concentrations determined from counts at the 5 μm size threshold for

chemically treated milk samples have been plotted against corresponding results obtained after centrifugal separation. In both methods determinations on duplicate samples were made and mean results are given.

Two results (X) showing exceptionally high ordinate values may be observed; incomplete fat dispersal following chemical treatment is thought to be the reason for these large deviations. The two sets of counts have been related by fitting linear

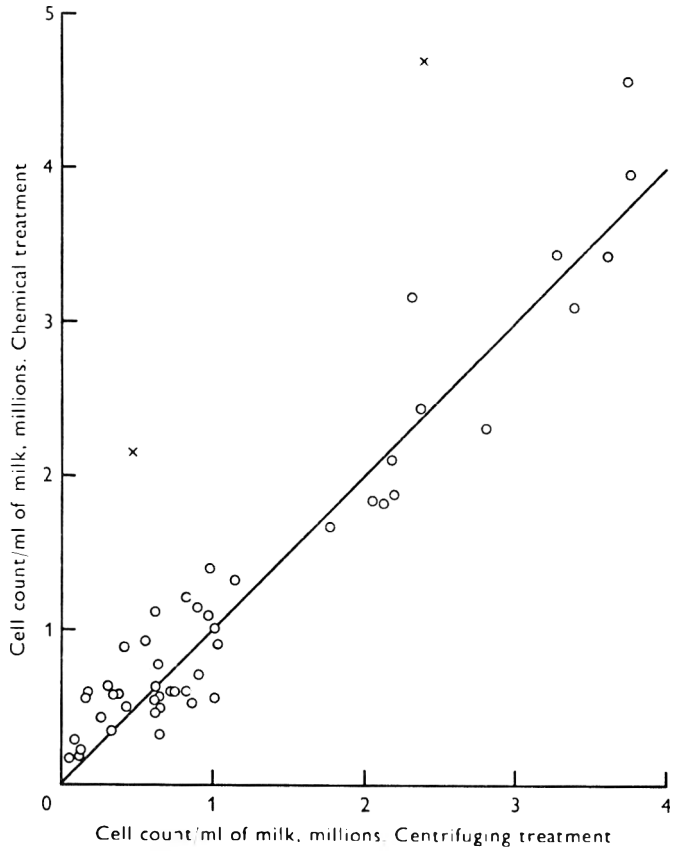

Fig. 5. Relation between cell counts obtained following the chemical and centrifuging methods of isolating the cells.

regression lines although there was a tendency for the chemically treated samples to yield higher counts in the region of low cell concentrations. The slopes of the linear regression lines calculated with and without the 2 particular results (X) were found to be 1·004 (S.E. ± 0·066) and 0·974 (S.E. ± 0·042), respectively. Since neither slope differed markedly from unity and the intercepts of the regression lines on the ordinate did not differ significantly from zero, a straight line of unit slope has been drawn through the origin to represent the least squares regression. Agreement between duplicate tests was closer, though not significantly so, after centrifugal separation (S.D. $\pm 0{\cdot}078 \times 10^6$/ml) than after chemical treatment (S.D. $\pm 0{\cdot}096 \times 10^6$/ml).

Comparison of surface active agents. Fifteen of the milks were also tested using the surface active liquid Lissapol NXP, a liquid less viscous and easier to dispense than

Witopal CO. The calibration constant of the counter did not need to be changed. As Fig. 6 shows, the difference between the cell counts obtained after Witopal CO and Lissapol NXP treatments was small and it is concluded that Lissapol NXP would be a suitable substitute for Witopal CO.

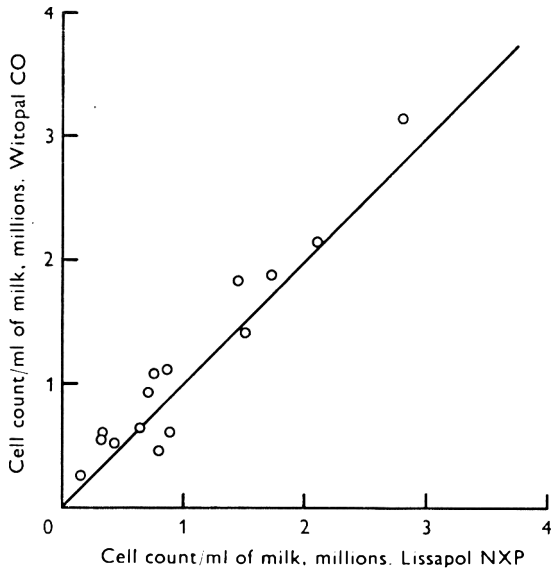

Fig. 6. Comparison of cell counts after using Witopal CO with corresponding results using Lissapol NXP.

CONCLUSIONS

In the present experiments the chemical procedure of Tolle *et al.* (1966) for preparing milk samples for electronic counting generally gave good results. Cell concentrations determined from counts at a 5 μm threshold size after the chemical treatment agreed well with results following centrifugal separation, though there was a tendency for relatively higher values to be obtained for milks of low cell content. An unfortunate feature of the chemical treatment that other workers have also noticed was the occasional occurrence of erratic and, in particular, exceptionally high counts with respect to corresponding microscope counts. The need to prepare a special electrolyte solution and to pretreat the milks with formalin also detracts from the method.

The advantage of the chemical procedure is the simplicity of technique and equipment. Though no attempt was made to experiment with large numbers of samples, it is apparent that with a suitably organized laboratory, one Coulter counter could be used to full capacity to provide a testing rate of about 60 samples/h. However, the centrifuging technique would seem capable of an equal rate of testing (Cullen, 1967).

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Factors affecting vacuum within the teatcup liner during milking

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Summary. Factors associated with the milking cluster and the long milk tube which cause a diminished or a fluctuating vacuum within the liner of the teatcup assembly during simulated milking with an artificial udder fell into 3 groups. 1. Lower milk flow rates and admission of air at the clawpiece greatly decreased vacuum fluctuation within a pulsation cycle and increased the mean level of vacuum in the liner during that part of the cycle in which milk was flowing from the teat. 2. Larger bore of the short milk tube connecting the liner to the clawpiece, pulsation of the teatcup liners in pairs instead of all 4 together, and larger volume of the clawpiece bowl in the main decreased fluctuation in vacuum within the liner. 3. Larger bore and shorter length of the long milk tube connected to the clawpiece and decreased height above the outlet of the clawpiece to which the milk was raised mainly increased the mean vacuum in the liner during that part of the cycle in which milk was flowing from the teat.

In a milking experiment with 75 cows mean overall fluctuations in milking vacuum within the liner in a pulsation cycle at peak flow were 11·5 and 4·8 inHg and corresponding mean levels of vacuum during that part of the cycle when milk was flowing from the teat were 11·75 and 13·0 inHg. There were no differences in milking performance of practical consequence.

The pressure conditions in the teatcup liner of a milking machine cluster during milking affect the rate of milking and may be associated with the incidence of mastitis. The influence of air admission at the clawpiece on mean milking vacuum and vacuum fluctuation in the teatcup liner, and the effect of the height to which milk is elevated from the clawpiece to the receiving vessel, with and without air admission, on milking rate, vacuum level, and pulsation ratio have already been described (Thiel, Clough & Akam, 1964; Clough, Thiel, Akam & Westgarth, 1965).

The results from these milking experiments suggested that the internal dimensions of all the basic components of a milking unit through which milk flows from the teatcup liner to the receiving vessel (where constant vacuum level was maintained throughout milking) could affect vacuum conditions in the teatcup liners.

Much of the investigation of combinations of factors influencing vacuum within the teatcup liners in the present paper was done with the aid of an apparatus with artificial teats to avoid the difficulties of short duration of constant flow rate in a milking and of differing flow rates from cow to cow.

The results of these experiments were used in setting up 2 milking machine

installations in a herringbone milking parlour with specified combinations of components which produced 'high' and 'low' levels of milking vacuum stability during milking. The milking properties of these machines were then compared.

METHODS

To simulate milking conditions with an artificial udder, the main requirements were constant flow rate at various preset levels and, within each pulsation cycle, that flow should cease when the teatcup liner was half closed and begin again when the liner next reached the half open position. The valve described in Fig. 1 was found

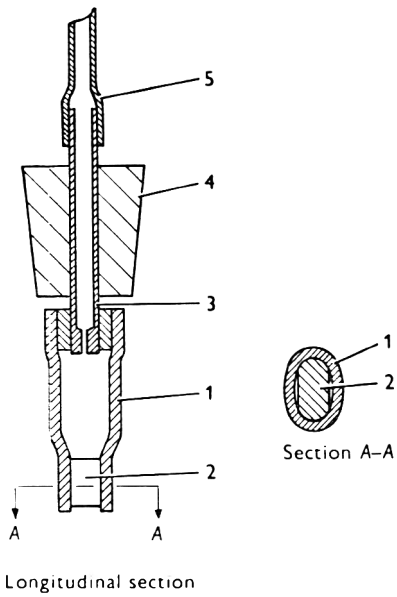

Fig. 1. Diagram of artificial teat. (1) Soft rubber tube 2 in. long × 0·75 in. O.D. × 0·50 in. I.D.; (2) brass plug, 0·5 in. vertical length × 0·625 in. wide × 0·323 in. thick, with semi-cylindrical vertical ends (note that the length of the periphery of the plug is 0·050 in. longer than the circumference of the bore of the rubber tube into which it is fitted); (3) brass orifice tube 2·5 in. long 0·31 in. diam., drilled 0·19 in. bore except for 0·25 in. of the lower end which was drilled various bores to give the required flow rates; (4) rubber bung to suit the liner used; (5) rubber tube 8 in. long, 4 of which connected a set of artificial teats to a constant head vessel (1-gall. bucket with an outlet at mid-height).

to open and close under the influence of liner-wall movement. Several sets of orifice tubes varying in bore from 0·06 to 0·12 in. gave a range of flow rates through the experimental cluster of about 5–15 lb/min. In comparisons of water at room temperature and milk at 39 °C used within a few minutes of milking, it was found that flow-rate was about 5% higher with milk than with water with any given bore of orifice tube, but no consistent differences in vacuum recordings within the liner or in the clawpiece were detected.

Some factors were held constant during the experiments. Vacuum to the pulsator and in the milk jar to which the long milk tube was attached was 15 inHg $^{+0\cdot2}_{-0\cdot0}$; one type of liner was used (Alfa Laval extruded liner no. 20003 B); pulsation

rate was 60 c/min with a constant wave-form of pressure change in the pulsation chamber (see, for example, Fig. 2).

The methods of pressure measurement were those described by Thiel *et al.* (1964). Instead of the ciné photographic method of recording liner-wall movement described by those authors, 2 metal fingers lightly bearing on the liner from opposite sides varied electrical resistances in accordance with their positions, and thus permitted simultaneous direct recording of liner-wall position and pressures. Because of the irregular nature of the cyclical changes in vacuum inside the liner under the teat and in the clawpiece (designated milking vacuum within the liner and in the clawpiece) the following arbitrary values only were used to characterize the curves: minimum milking vacuum as the liner closed; maximum milking vacuum during the part of the cycle that the liner was more than half open; mean milking vacuum while the liner was more than half open; and overall vacuum fluctuation defined as the difference recorded in a pulsation cycle between the minimum vacuum which generally occurred as the liner closed and the maximum vacuum which occurred as the liner opened.

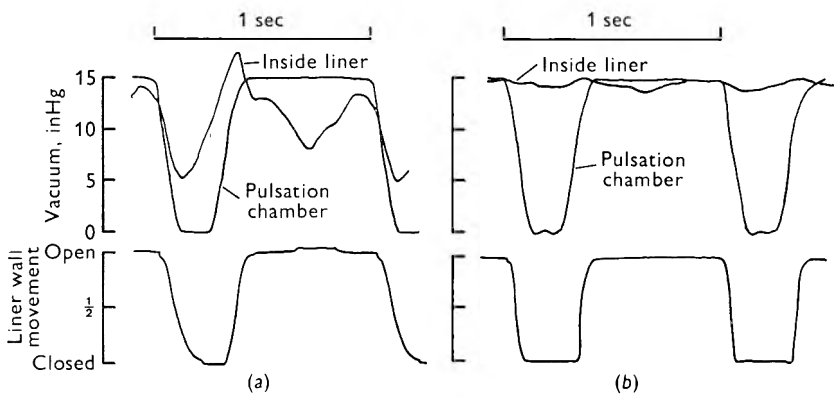

Fig. 2. Examples of large and small changes in milking vacuum within a pulsation cycle. (*a*) Flow-rate, 9·4 lb/min; water elevated 5 ft; length of long milk tube, 6 ft; volume of clawpiece bowl, 20 ml. (*b*) Corresponding figures 5·0 lb/min; 0 ft; 3 ft; 460 ml.

FACTORS AFFECTING MILKING VACUUM

The factors investigated were flow-rate, length of the long milk tube, volume of the chamber of the clawpiece, height to which liquid was raised above the outlet of the clawpiece to the entry into the milk jar, rate of air admission to the clawpiece, bore of the long milk tube, bore of the short milk tubes joining the liners to the clawpiece and the bores of the nipples on the clawpiece, pulsating the liners in pairs and all 4 together, the degree of tension in the liner and the volume of the liner occupied by the teat. The first 4 factors were examined by means of a factorial design of experiment; the remainder were examined separately after the findings of the first experiment had indicated a length of long milk tube, height of elevation and clawpiece volume likely to be acceptable in a practical milking machine for use in a 2-stall/unit herringbone parlour.

Flow-rate, length of long milk tube, clawpiece volume, and height of elevation

Constant factors were bore of long milk tube, 0·5 in.; air admission at the clawpiece, 0·25 ft³/min of free air entering the clawpiece with a pressure difference across the air admission hole of 15 inHg; liners pulsated in pairs; bore of short milk stems, about 0·25 in.

The following variable factors were tested at the levels indicated:

Elevation of the liquid above the outlet of the clawpiece, E	−1, 0, 2, 3, 5 ft
Volume of clawpiece chamber, V	20, 116, 460 ml
Length of long milk tube, L	3, 5, 6, 8, ft
Flow-rate, F	5·1, 9·4 lb/min

The factors could not be tested in a single experiment in a completely factorial arrangement because of the impossibility of combining high milk elevation with short length of the long milk tube. The range of conditions was covered in 2 experiments, each of which was factorial:

Expt 1. $E(-1, 0, 2, 3, 5$ ft$) \times V(20, 116, 460$ ml$) \times L(6, 8$ ft$) \times F(5{\cdot}1, 9{\cdot}4$ lb/min), i.e. single replicate, total 60 observations

Expt 2. $E(-1, 0, 2$ ft$) \times V(20, 116, 460$ ml$) \times L(3, 5, 6, 8$ ft$) \times F(5{\cdot}1, 9{\cdot}4$ lb/min), i.e. single replicate, total 72 observations

Typical records showing the smallest and largest fluctuations in milking vacuum within a pulsation cycle, and liner-wall movement, are reproduced in Fig. 2.

Analyses of variance of mean vacuum while the liner was more than half open, maximum vacuum in the same period of the pulsation cycle, and minimum vacuum as the liner closed, all taken from the records of pressure change within the liner, were calculated separately for each experiment. Standard errors of treatment means and the corresponding 5% least significant differences (L.S.D.) were derived from the 4-factor interaction mean squares with 8 degrees of freedom (expt 1) and 12 degrees of freedom (expt 2).

The effects of the various factors on mean milking vacuum at the teat while the liner was open are summarized in Table 1. Significant effects were as follows. Increasing elevation and increasing length of the long milk tube both decreased mean vacuum: their effects did not interact. Under the particular conditions of these experiments an increase in elevation from −1 to 5 ft gave an average depression of 1·6 inHg in mean vacuum, and increasing the length of the long milk tube from 3 to 8 ft depressed the mean vacuum by 0·8 inHg. Similarly, increasing flow-rate from 5·1 to 9·4 lb/min depressed the mean vacuum, on average, by 0·9 inHg. There was some indication of a small increase in mean vacuum of about 0·2 inHg when the volume of the clawpieee was increased from 20 to 460 ml but significance was achieved only in expt 2. Interactions between the effects of these factors on mean vacuum appear to be small and were not clearly established in both experiments.

The effects of the 4 factors on maximum vacuum (liner opening) and minimum vacuum (liner closing) are shown in Table 2 and may be considered together in their general effect on over-all vacuum fluctuation. Increasing the flow-rate from 5·1 to 9·4 lb/min, on average increased maximum vacuum slightly attaining a 5% level of

significance in expt 2 but only a 10% level in expt 1, and decreased minimum vacuum markedly so that the greater flow increased vacuum fluctuation by about 4 inHg with most of the effect associated with decrease in minimum vacuum. With increasing elevation both minimum and maximum vacuum decreased, and the net effect on over-all vacuum fluctuation was relatively small. No simple average effect

Table 1. *Mean milking vacuum while liner was open*

						5% L.S.D.
Elevation, ft	−1	0	2	3	5	—
Mean vac., inHg						
Expt 1	13·8	13·7	13·0	12·8	12·2	0·19
Expt 2	14·0	13·8	13·2	—	—	0·06
Length of tube, ft	3	5	6	8	—	—
Mean vac., inHg						
Expt 1	—	—	13·4	12·8	—	0·12
Expt 2	14·0	13·7	13·8	13·2	—	0·07
Flow-rate, lb/min	5·1	9·4	—	—	—	—
Mean vac., inHg						
Expt 1	13·6	12·6	—	—	—	0·12
Expt 2	14·1	13·3	—	—	—	0·5
Vol. clawpiece, ml	20	116	460	—	—	—
Mean vac., inHg						
Expt 1	13·0	13·1	13·2	—	—	0·14
Expt 2	13·5	13·8	13·8	—	—	0·06

Table 2. *Maximum vacuum (liner open) and minimum vacuum (liner closed)*

						5% L.S.D.
Elevation, ft	−1	0	2	3	5	—
Expt 1. Max. vac., inHg	15·6	15·5	14·9	14·8	14·5	0·35
Min. vac.	9·6	9·2	8·9	9·0	8·1	0·57
Expt 2. Max. vac.	15·8	15·8	15·3	—	—	0·26
Min. vac.	9·5	9·3	8·8	—	—	0·32
Length of tube, ft	3	5	6	6	—	—
Expt 1. Max vac., inHg	—	—	15·5	14·7	—	0·22
Min. vac.	—	—	8·8	9·1	—	0·36
Expt 2. Max. vac.	16·0	15·9	15·8	14·9	—	0·30
Min. vac.	9·7	8·7	9·0	9·5		0·37
Flow-rate, lb/min	5·1	9·4	—	—	—	—
Expt 1. Max. vac., inHg	15·0	15·2	—	—	—	0·22
Min. vac.	11·0	6·9	—	—	—	0·36
Expt 2. Max. vac.	15·5	15·8	—	—	—	0·21
Min. vac.	11·2	7·2	—	—	—	0·26
Vol. clawpiece, ml	20	116	460	—	—	—
Expt. 1. Max. vac., inHg	16·7	14·6	13·9	—	—	0·27
Min. vac.	7·3	9·1	10·4	—	—	0·44
Expt 2. Max. vac.	17·0	15·4	14·5	—	—	0·26
Min. vac.	7·3	9·6	10·8	—	—	0·32

of length of the long milk tube on minimum vacuum emerged, although increasing the tube length from 3 to 8 ft decreased maximum vacuum by about 1 inHg, the net effect being a decrease in over-all vacuum fluctuation at long tube lengths. Increasing the clawpiece volume from 20 to 460 ml decreased maximum vacuum and increased minimum vacuum, so that the larger the clawpiece volume the smaller was over-all vacuum fluctuation.

A significant interaction effect between clawpiece volume and flow rate on maximum vacuum is noteworthy and is shown in Table 3. Increasing volume from 20 to 460 ml depressed maximum vacuum less at the lower flow rate (average depression, 3·6 inHg). No such interaction was obtained for minimum vacuum whose increase with increased volume of clawpiece (3·3 inHg) was independent of flow rate. Consequently, the decrease in over-all vacuum fluctuation with increased volume averaged 5·0 inHg at the lower flow rate but 6·9 inHg at the higher rate.

Table 3. *Interaction of flow rate and volume of clawpiece on maximum vacuum (liner open), inHg*

	Flow rate, lb/min		
Volume, ml	5·1	9·4	Mean
		Expt 1	
20	15·9	17·4	16·7
116	14·8	14·5	14·6
460	14·2	13·5	13·9
Mean	15·0	15·2	15·1
		Expt 2	
20	16·4	17·6	17·0
116	15·2	15·5	15·4
460	14·7	14·3	14·5
Mean	15·5	15·8	15·6

Other factors affecting milking vacuum considered in relation to a practical form of milking machine for use in a herringbone parlour

From the above analyses it appeared likely that means of limiting changes in milking vacuum within the liner might be fairly readily found for bucket-milking equipment and low-level machines in parlours. However, in 2-stall/unit parlours with lengths of long milk tube probably greater than 5 ft to allow the milking cluster to be moved from one side of the operator's pit to the other, and involving considerable elevation of the milk to an overhead pipeline or a milk jar placed for accurate reading of milk volume, the problem seemed more difficult. Further study of factors influencing milking vacuum at the teat using simulated milking was therefore confined to equipment in which the entry to the milk jar was 3 ft above the clawpiece outlet, and a 6 ft length of long milk tube was used. It was judged that these dimensions would not be unduly restrictive in designing milking equipment for a herringbone parlour with a central overhead milk transport pipe.

Bore of the short milk tubes. It was apparent from preliminary work that considerable pressure differences could exist between the space inside the liner and the chamber of the clawpiece, indicating a restricting effect of the short milk tubes and clawpiece nipples. Under the conditions of measurement given in the legend to Fig. 3, it could be seen from the pressure traces that the greatest pressure differences from the liner to the clawpiece occurred when the liner wall was moving and especially when closing. Little difference in mean milking vacuum during the time the liner was more than half open occurred between the 2 ends of the short milk tube at all flow rates (upper curves in Fig. 3). Over-all fluctuations in milking vacuum were similar in

the liner and the clawpiece only when the bores of the short milk tubes and clawpiece nipples were increased to 0·44 in., and when the liners were pulsated in pairs rather than all 4 together.

With the smallest bore of short milk tubes (0·25 in.) over-all fluctuations measured inside the liner were large and increased substantially with flow rate. In the clawpiece, fluctuations were smaller and little influenced by flow rate, thus indicating marked restriction by the short milk tube. With milk tubes of 0·38 in. bore, over-all vacuum fluctuations in the liner were reduced at low flow rate but remained high at the higher flow rates. There was also a substantial increase in the range of over-all

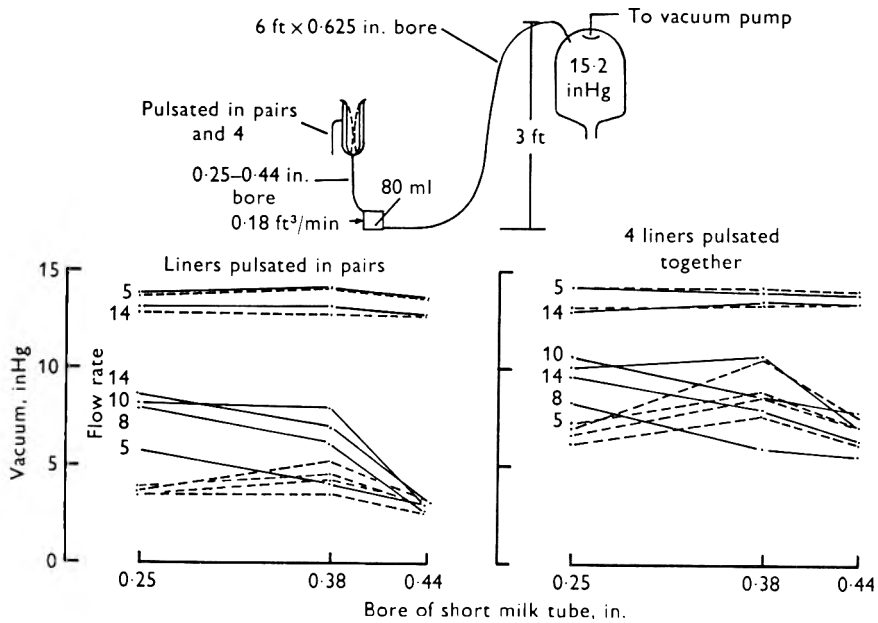

Fig. 3. Effects of bore of short milk tube and pulsation of liners in pairs or all 4 together on over-all vacuum fluctuations in a pulsation cycle (lower sets of curves) and on mean milking vacuum when the liners were more than half open (upper sets of curves). Dotted lines, pressures measured in the clawpiece; full lines, pressures measured inside the liner. Figures indicate the flow rate in lb/min. Simulated milking using water elevated 3 ft; length of long milk tube 6 ft, bore 0·625 in; clawpiece volume 80 ml; air admission rate 0·18 ft³/min of free air; volume change of each liner on opening and closing 22 ml.

fluctuations in the clawpiece. With a further increase in bore of the short milk tubes to 0·44 in., the range of over-all vacuum fluctuations over the range of flow rates was much reduced both inside the liner and in the clawpiece. It seems that only with a generous bore of the short milk tubes will milk in transit be sufficiently reduced in quantity or occupy a sufficiently small proportion of the passage ways to avoid noticeable impediment to air movement as the liners change in volume.

Pulsating liners in pairs alternately or all 4 together, and change in volume of each liner. Since minimum vacuum measured in the liner is causally related to decrease in volume of the cluster as the liners close, and maximum vacuum to increase in volume on opening (Thiel *et al.* 1964), it might be expected that over-all vacuum fluctuation would be less when liners are pulsated in pairs compared with all 4 together. The data

summarized in Fig. 3 show that this was so at all flow rates and all bores of short milk tubes. Further data are given in Fig. 4, together with comparative effects of volume changes/liner of 22 and 14 ml, representing a small and a large volume of teat entering

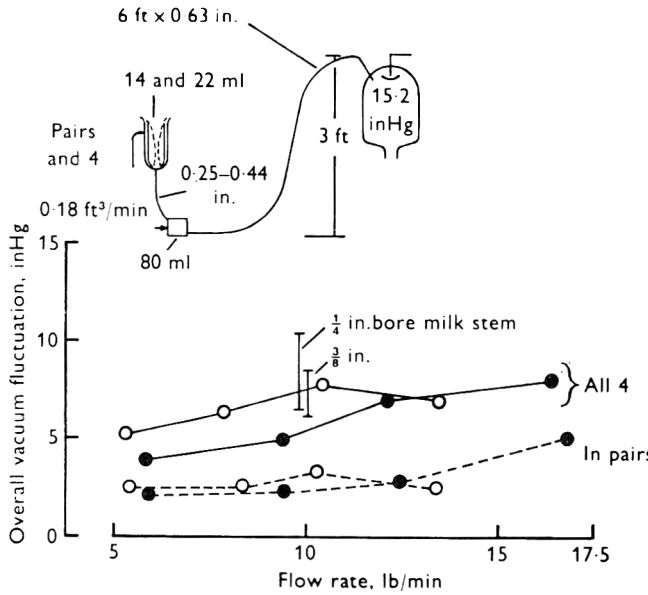

Fig. 4. Effect of pulsation of liners in pairs or all 4 together, and volume change/liner, on over-all milking vacuum fluctuations. ○, 22 ml change in volume/liner; ●, 14 ml. Simulated milking using water elevated 3 ft; length of long milk tube 6 ft, bore 0·625 in; clawpiece volume 80 ml; air admission rate 0·18 ft³/min of free air; bore of short milk tubes 0·44 in. (corresponding ranges of values for bores of 0·38 and 0·25 in. are shown for a flow rate of 10 lb/min).

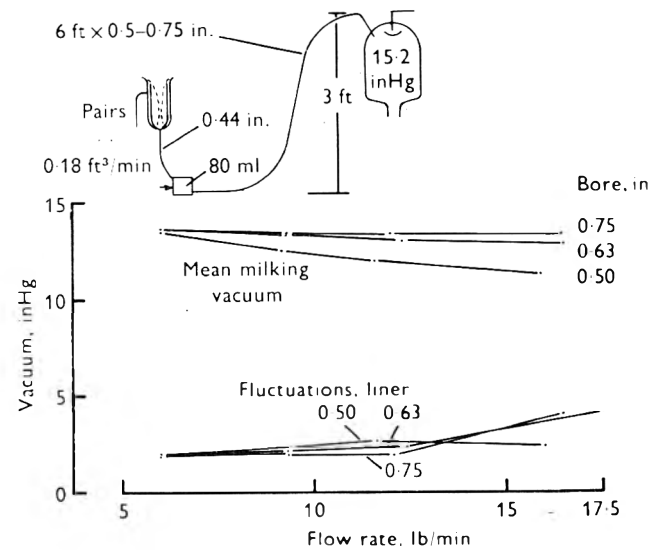

Fig. 5. Effect of bore of a long milk tube 6 ft in length on mean milking vacuum (upper curves) and over-all vacuum fluctuation (lower curves) measured inside the liner. Figures indicate bore in inches. Simulated milking using water elevated 3 ft; clawpiece volume 80 ml; air admission rate 0·18 ft³/min of free air; liners pulsated in pairs; bore of short milk tubes 0·44 in.

the liner. Type of pulsation had much the greater effect. When slack liners and the same liners under tension were compared, no effect on over-all fluctuation was detected. When liners were pulsated in pairs with opening of one pair occurring simultaneously with closing of the other, overall fluctuation was further reduced to less than 1 inHg. This, however, could be achieved in practice only by sacrificing the increased milking rate obtained with wide ratio pulsation.

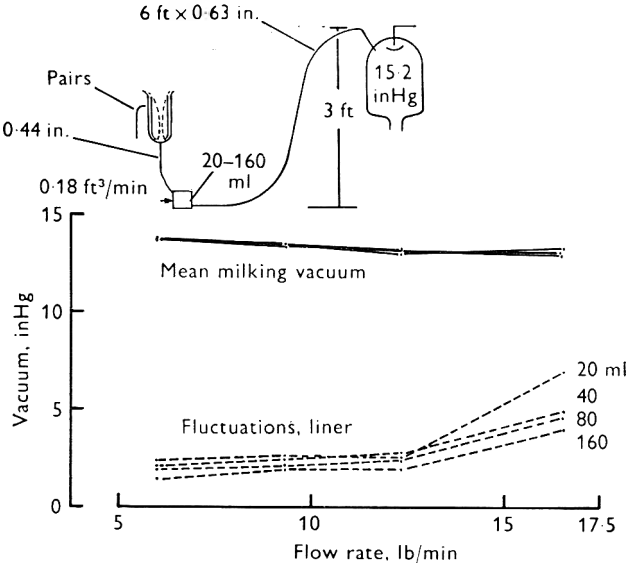

Fig. 6. Effect of volume of the clawpiece chamber on mean milking vacuum (upper curves) and over-all vacuum fluctuation (lower curves) measured inside the liner. Figures indicate clawpiece volume in ml. Simulated milking using water elevated 3 ft; air admission rate 0·18 ft³/min of free air; liners pulsated in pairs with volume change/liner of 14 ml; bore of short milk tubes 0·44 in; length of long milk tube 6 ft, bore 0·63 in.

Bore of the long milk tube. Mean milking vacuum during the part of the pulsation cycle in which the liner was more than half open moved closer to the nominal milking machine vacuum of 15·0 inHg when the bore of a 6 ft length of rubber long milk tube was increased from 0·5 to 0·63 in. (Fig. 5, upper curves). Further increase in bore to 0·75 in. gave a small additional increase at the higher flow rates. Over-all vacuum fluctuations were little affected by the bore of the long milk tube (Fig. 5, lower curves) under the conditions of the experiment referred to in the legend.

Volume of the clawpiece chamber. An 8:1 change in volume of the clawpiece chamber had no detectable effect on mean milking vacuum during the part of the pulsation cycle in which the liner was more than half open, over a range of flow rates from 6 to 16 lb/min (Fig. 6, upper curves). Increasing the volume of the clawpiece somewhat reduced over-all vacuum fluctuation with flow rates up to 12·5 lb/min, and had more marked effect at 16 lb/min (Fig. 6, lower curves). This small effect of increasing clawpiece volume on over-all vacuum fluctuation is in contrast with the large effect found in the earlier factorial experiment. The explanation is that reduction of large transient pressure differences between the inside of the liners and the bowl of the clawpiece, by increasing the bore of the short milk tubes and by pulsating liners in pairs instead of all 4 together, substantially reduced over-all vacuum fluctuation in the

clawpiece. The usefulness of a large volume for damping purposes was correspondingly reduced.

Rate of air admission at the clawpiece. With no air admission at the clawpiece there was a large over-all fluctuation in milking vacuum within the teatcup liner. Mean milking vacuum was severely reduced during the part of the pulsation cycle that the liner was more than half open. At all rates of air admission from 0·1 to 0·5 ft³/min of free air, over-all vacuum fluctuations were similar and much reduced compared with the rate with no air admission. Also levels of mean milking vacuum were considerably raised by air admission, but were similar for rates from 0·1 to 0·25 ft³/min of free air. At 0·5 ft³/min there was some indication of depression in mean milking vacuum at high flow rates (Fig. 7).

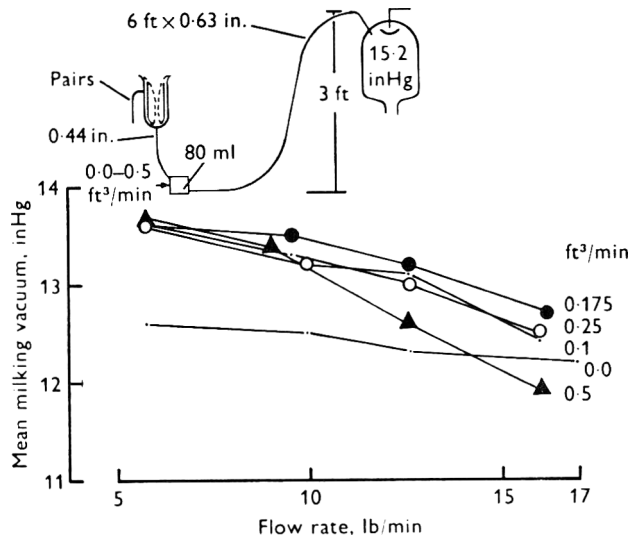

Fig. 7. Effect of rate of air admission at the clawpiece on mean milking vacuum during that part of the pulsation cycle when the liner was more than half open. Figures indicate air admission rate in ft³/min of free air. Simulated milking using water elevated 3 ft; long milk tube 6 ft × 0·63 in. bore; volume of clawpiece 80 ml; bore of short milk tubes 0·44 in; liners pulsated in pairs.

EFFECT OF TWO LEVELS OF VACUUM FLUCTUATION ON MILKING PERFORMANCE

A practical form of milking machine often used at the present time in herringbone parlours in Great Britain is characterized by having 1 milking unit for 2 stalls and calibrated milk jars placed centrally in the operator's pit with the milk entry to the jar usually about 6 ft above the operator's floor level. The influence of 'high' versus 'low' levels of vacuum fluctuation within the liner under the teat on milking performance of a 5-unit machine of this type was measured by peak flow rate in any 1 min; machine time, machine yield, and hence machine rate from application of the unit to stripping point (less than 0·1 lb in 1 min); and yield of machine strippings.

A herd of 75 Friesian cows of various lactation ages and stages of lactation were milked at 4 consecutive morning milkings using alternately the machine conditions detailed in Table 4 to give 'high' and 'low' vacuum fluctuations. On each occasion the same 17 cows were milked by the centre unit of the 5, which was equipped for recording pressure changes within the liner to obtain a measure of 'high' and 'low'

vacuum fluctuation (HVF and LVF). Typical records of vacuum when milking a cow with a moderately high peak flow rate are given in Fig. 8.

Summaries of the main data are given in Tables 5 and 6. It may be seen from the treatment means in Table 5 that although the 'high' vacuum fluctuations were more than double the 'low' fluctuations, of the various measures of milking performance only peak flow rate was affected. Peak flow rate with 'high' fluctuations was about 7% less than with 'low' fluctuations, a difference too small to affect significantly machine time or machine rate. Stripping yields were unaffected. The proportion of

Table 4. *Milking machine conditions in a herringbone parlour giving 'high' and 'low' over-all vacuum fluctuations within the liner*

Machine condition	High fluctuations	Low fluctuations
Bore of short milk tube including clawpiece nipple, in.	About 0·31	0·44
Type of pulsation	All 4 teatcup liners together	In pairs
Elevation of milk above clawpiece outlet, ft	5	3
Bore of long milk tube, in.	0·5	0·625
Length of long milk tube, ft	9	6
Volume of clawpiece bowl, ml	20	116

Other common conditions: vacuum in milk jar, 15·2 inHg $^{+0\cdot0}_{-0\cdot5}$; air admission at clawpiece, 0·18 ft³/min of free air; % liner open, 70; pulsation rate, 60 c/min; cluster weight, 6·75 lb.

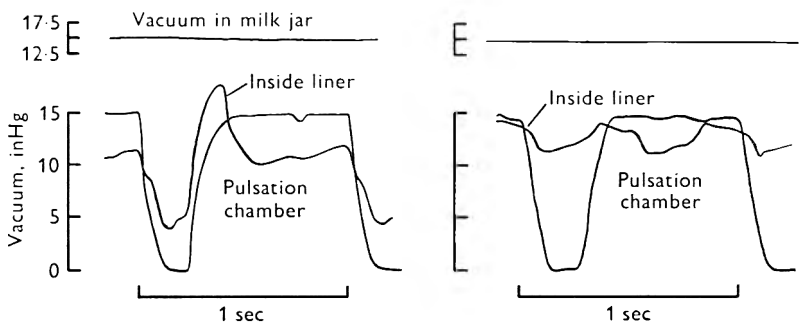

Fig. 8. Vacuum records showing typical 'high' and 'low' fluctuations in milking vacuum. Cow 45, peak milk flow rate 8·75 lb/min with 'high' fluctuations and 9·5 with 'low' fluctuations.

the pulsation cycle for which the liner was more than half open and milk was flowing was inferred for the group of 17 cows from simultaneous recordings of pressures in the pulsation chamber and within the liner under the teat. The half open and half closed positions of the liner were taken to occur when pressure difference across the liner was 3·5 inHg. With 'high' and 'low' fluctuations, the liner was more than half open on average for 70·4 and 69·1% of the cycle. For each recording a mean value of milking vacuum was estimated graphically for the part of the cycle during which the liner was more than half open. For the 17 cows, the averages of these mean values were 11·75 inHg with 'high' fluctuations and 13·0 inHg with 'low' fluctuations. Since the duration of flow appeared constant in a cycle, the small depression of peak flow rate

of 7 % with 'high' vacuum fluctuations might be accounted for by this depression in mean milking vacuum.

Table 6 gives the between-cow regression equations relating each of the milking characteristics (stripping yield, machine time, machine rate, and peak flow rate) with

Table 5. *Milking characteristics with 'high' and 'low' over-all vacuum fluctuations (HVF and LVF)*

	Treatment means			S.E. of difference
	HVF	LVF	Difference	(74 D.F.)
All 75 cows				
Machine yield, lb/milking	17·96	18·04	−0·08 NS	0·090
Stripping yield, lb/milking	0·55	0·60	−0·05 NS	0·038
Machine time, min/milking	4·22	4·25	−0·03 NS	0·098
Peak flow rate, lb/min	7·27	7·81	−0·54***	0·100
17 cows only				(16 D.F.)
Peak flow rate, lb/min	9·01	9·74	−0·73*	0·249
Overall vacuum fluctuation, inHg	11·53	4·82	6·71***	0·423

NS, not significant; * significant at 5 % level; *** significant at 0·1 % level.

Mean milking vacuum within the liner during the part of the cycle that the liner was more than half open: HVF 11·75 inHg; LVF 13·0 inHg (17 cows only).

Table. 6. *Regressions relating milking characteristics, y, with yield, x (lb/milking) under conditions of 'high' and 'low' over-all vacuum fluctuations (HVF and LVF)*

y	$y = a + bx$		S.E. of *b*
Between 75 cows			
Stripping yield, lb/milking			(73 D.F.)
HVF	$y =$ 0·25	+0·017**x	0·005
LVF	0·28	+0·018**	0·006
HVF–LVF†	−0·02	−0·002 NS	0·006
Machine time, min/milking			
HVF	1·90	+0·129***	0·021
LVF	1·79	+0·137***	0·019
HVF–LVF	0·10	−0·008 NS	0·013
Machine rate, lb/min			
HVF	2·74	+0·098***	0·022
LVF	2·78	+0·091***	0·020
HVF–LVF	0·04	+0·006 NS	0·016
Peak flow rate, lb/min			
HVF	3·44	+0·214***	0·034
LVF	3·20	+0·255***	0·038
HVF–LVF	0·36	−0·050**	0·015
Between 17 cows only			
Peak flow rate, lb/min			(15 D.F.)
HVF	5·81	+0·135 NS	0·118
LVF	5·11	+0·194 NS	0·133
HVF–LVF	1·92	−0·111*	0·048
Vacuum fluctuation, inHg			
HVF	7·82	+0·156*	0·072
LVF	0·38	+0·186*	0·078
HVF–LVF	8·00	−0·054 NS	0·093

Significance levels: NS ($P > 0·05$), * ($P < 0·05$), ** ($P < 0·01$), *** ($P < 0·001$).

† HVF–LVF: the dependent variate is the mean difference between a cow's milking characteristic under HVF and LVF conditions. The independent variate, x, is the cow's yield averaged over HVF and LVF conditions.

yield. The usual positive correlations were obtained under conditions of either 'high' or 'low' vacuum fluctuations. The table also shows that within-cow regressions relating the effect of increased vacuum fluctuation on these milking characteristics with yield are not significant except in the case of peak flow rate, i.e. although the depression in peak flow rate due to 'high' vacuum fluctuation averaged 0·54 lb/min among the 75 cows (Table 5), the high yielders suffered more marked decreases than did the low yielders.

DISCUSSION

If milking units are set as for milking with the mouths of the liners uppermost but closed with stoppers, there is so little resistance to movement of air in and out of the liners as their internal volumes change on opening and closing, that no change in vacuum within the liners is usually detected. An equally stable vacuum during milking may be achieved if milk drains away under gravity in such a manner that no air way in the cluster is ever substantially filled at any cross-section with milk. If, however, drainage is less effective than this or, as commonly occurs, milk is elevated from the clawpiece and transported some distance, changes in vacuum within the teatcup will repeat during each pulsation cycle. The causes are readily understood. First, work will be done in elevating the milk, in accelerating it to its final velocity, and in overcoming frictional resistance to flow. The only source of force available for these purposes is the pressure difference between the cluster and the outlet end of the long milk tube, i.e. a lowered vacuum in the cluster will occur. Secondly, milk in transit through the milking unit can very readily impede the free movement of air in and out of the liners in each pulsation cycle. Rapid increase in pressure difference from the liners to the outlet of the long milk tube, commensurate with rate of change of pressure in the pulsation chambers, results in violent movement of the restraining milk. Thiel *et al.* (1964) found that oscillatory movement at the natural frequency of the system may occur under extreme conditions of flooding of the cluster, resulting in cyclical pressure change as high as 20 inHg.

Factors in the present study influencing vacuum changes within the liners of a milking unit may be grouped into (i) those influencing mean milking vacuum during the part of the pulsation cycle in which milk is flowing, as well as influencing over-all fluctuation in vacuum in a cycle; (ii) those predominantly influencing the extent of vacuum fluctuation; and (iii) those predominantly affecting mean milking vacuum.

(1) *Factors influencing mean milking vacuum level as well as over-all vacuum fluctuation.* Increasing milk flow rate so giving more milk in transit to impede free movement of air, greatly increased vacuum fluctuation, and by requiring more energy for milk transport decreased mean milking vacuum. Admission of air at the clawpiece markedly reduced milk in transit and had a dramatic effect in moderating vacuum fluctuation, practically eliminating vacuum within the liner in excess of the nominal milking machine vacuum. In addition, when milk was elevated above the outlet of the clawpiece, the mean milking vacuum was increased by air admission. Increasing the rate of admission over the range 0·1–0·25 ft^3/min of free air had only a small effect on mean milking vacuum. Presumably the beneficial effects of minimizing the quantity of milk in transit through the unit and reducing the mean density of fluid in transit were offset by the general increase in fluid velocities which occurred with

increasing rate of air admission. At 0·5 ft^3/min of free air there was some evidence of decline in mean milking vacuum. It would appear that with present methods of admitting air to the cluster the generally accepted rate of about 0·25 ft^3/min of free air is reasonable, as lower rates require small air admission holes which are difficult to keep clear, and higher rates induce some decline in vacuum within the liner for the part of the pulsation cycle during which milk is flowing from the teat.

(ii) *Factors predominantly influencing extent of vacuum fluctuation.* Next to admitting air at the clawpiece, increasing the bore of the short milk tube joining the liner to the clawpiece had the greatest effect in decreasing vacuum fluctuation. Nevertheless, a bore of 0·44 in., although adequate to suppress pressure differences between the interior of the liners and the bowl of the clawpiece when liners were pulsated in pairs, was inadequate when all 4 liners were pulsated together. Since volume change within the milk system of the cluster as the liners open and close is the cause of vacuum fluctuation, it is not unexpected that the smaller volume change, when liners pulsate in pairs or when teats penetrate deep into the liners, decreased markedly the extent of vacuum fluctuation. Once pressure differences between the interior of liners and the bowl of the clawpiece were reduced to negligible proportions, volume of the clawpiece bowl was of secondary importance, presumably because one of the main effects of a large volume is to apply damping to the system.

(iii) *Factors mainly influencing mean milking vacuum within the liner.* The mean milking vacuum within the liner during that part of the cycle during which the liner was more than half open was depressed by increasing length of the long milk tube and decreasing bore (both of which increase frictional losses) and increased elevation of milk above the outlet of the clawpiece.

The technique employing water from artificial tests was useful in comparing factors affecting milking vacuum within the liner. Results obtained with the apparatus when using water and freshly drawn milk were closely similar. However, it appeared when milking cows that a somewhat greater fluctuation in vacuum occurred than was expected from the simulated milking experiments.

While the present investigations give some indication of the relative effects of the different factors affecting milking vacuum inside the teatcup liner, there was no difference in milking performance of practical consequence with 'high' and 'low' over-all vacuum fluctuations within a pulsation cycle (mean over-all fluctuations of 11·5 and 4·8 inHg). Thus, it appears that the value of increased vacuum stability must continue to be judged by benefits such as improved animal health or lactational yield. Even so, a clear understanding of the physical basis of any proven ill effect of vacuum fluctuation might point to other cures than suppression with its attendant limitations on machine design.

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Reviews of the progress of Dairy Science

Section G. Deposits from whole milk in heat treatment plant—a review and discussion

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CONTENTS

INTRODUCTION

With the increasing use of ultra-high-temperature (UHT) milk treatment processes in which the milk is heated to temperatures above 100 °C, the deposition of solid material from the hot milk on plant surfaces has become of great practical importance. Severe cleaning problems are caused, and although new detergents are able to remove the deposits in most circumstances, manual cleaning of some components may be necessary. A more important effect is the possible limitation of the time for which a heat treatment plant can be operated without intermediate cleaning. Any limitation is economically undesirable, as it reduces the availability of expensive plant and increases the processing costs. The operating time may be restricted either through the deposits obstructing the flow passages in the heat exchanger, so causing excessive pressure drop, or through a reduction of the heat transfer coefficients for heating surfaces, so making it increasingly difficult to maintain the required processing temperature. If the deposits become detached by the milk flow, they may cause unsightly sediment in the finished milk.

Although deposit formation has become of such practical importance, comparatively little has been published on the subject and the processes involved are not understood. In this review, the available information bearing on the formation of deposits is surveyed, and possible mechanisms are considered which are in agreement with the published results. At the present stage of knowledge the mechanisms are largely hypothetical, but it is hoped that an interim discussion will stimulate new lines of thought, leading to benefits in practical heat treatment operations.

METHODS FOR EXPERIMENTAL INVESTIGATION

The relative scarcity of information on deposits and their formation may be partly due to the difficulties of experimental work. In a processing dairy, problems arise when thousands of gallons of milk have passed through the plant. The scale of the operation thus makes it impracticable to carry out true research studies during commercial processing. As an alternative, small heat exchangers with throughputs of about 10 gal/h have been used. With a tubular heat exchanger (Bell & Sanders, 1944), the deposit is inaccessible and its distribution and composition cannot be determined. Results must be inferred from pressure and temperature data. If a plate heat exchanger is used (Gynning, Thomé & Samuelsson, 1958), distribution and composition can be determined, but operating pressures are limited by the plate gasket characteristics. Even with these small heat exchangers, the number of tests which can be made is still limited, and is probably not more than 2/day. A laboratory method using a heated stainless steel tube immersed in milk has been used, but it has limitations (Burton, 1961). Recently a method has been developed which requires only a 350 ml sample of milk to give a result in about 1 h (Burton, 1965). This uses an electrically-heated platinum wire as the deposition surface, and has been shown to reproduce the differences between milks detected in a heat exchanger (Burton, 1966*a*). It seems very suitable for studying the differences in deposit formation resulting from changes in milk composition or changes in pretreatment, but it is not suitable for studying the relation between the deposit and the surface on which it is formed, as the deposition surface is of platinum rather than of the stainless steel which is almost universal in practice.

DEPOSIT DISTRIBUTION AND COMPOSITION

In most indirectly heated UHT processing plants, the deposit formation is greatest in the final heating section, where steam or pressurized hot water is the heating medium. In this section, the milk is heated from about 85 to 140 °C. Before considering the distribution and composition of the deposits formed, it is necessary to anticipate and to state the effect of preholding, which has a marked influence on the deposit picture and which will be discussed again later.

Bell & Sanders (1944) found that milk which had been preheld at temperatures above about 65 °C gave less pressure drop in a steam-heated tubular heat exchanger than did non-preheated milk. They proposed preholding as a practical means of reducing the amount of deposit formation, and their solution was widely adopted. However, since their experiments were with a tubular heater, they were unable to tell by visual examination why the improvement was obtained. Examination of plate heat exchangers has filled in some of the picture (Ito, Sato & Suzuki, 1962*a*, *b*; Lyster, 1965).

If the milk has not been preheld, but passes without delay from the earlier regenerative stages into the final heater, the distribution of deposit in the final heater is as shown diagrammatically in Fig. 1*a*. The maximum amount of deposits occurs at a relatively low temperature, perhaps about 100–105 °C, and the deposit then decreases to a comparatively small quantity at the heater outlet. The type of deposit

also changes throughout the heater. The lower temperature deposit (type A), which comprises the largest amount, is a soft, voluminous, curd-like material, white or cream in colour, which may overlay a harder base probably caused by over-heating at the heating surface. It is largely made up of protein (50–60 %), but also contains a substantial amount of mineral matter (30–35 %). The fat content is low (4–8 %) and variable.

In the higher-temperature part of the heating section, this deposit changes imperceptibly into a second (type B) which is brittle and gritty, and grey in colour except where it has been overheated at a surface. This deposit has a higher ash content than type A (about 70 %) and a lower protein content (15–20 %). The fat content is probably similar in the 2 types.

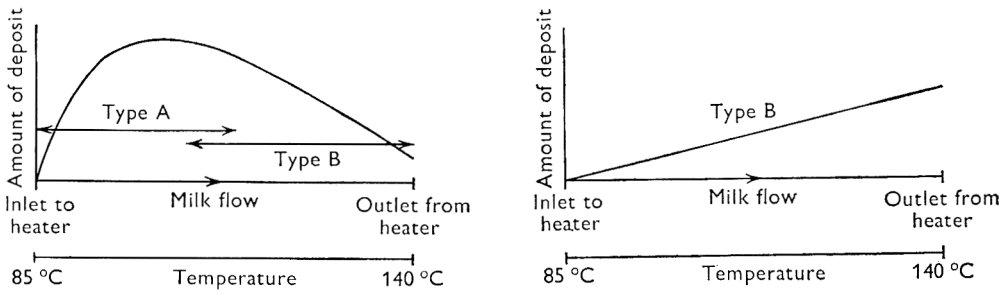

Fig. 1. (*a*) Distribution of deposits without preholding.
(*b*) Distribution of deposits with preholding.

When milk is not preheld so that this deposit picture results, plant operation is affected through the restriction of flow passages by the type A deposit in the early parts of the final heating section. This causes the pressure drop through the plant to increase, but not linearly with time. First there is a period during which the pressure change is comparatively slight: there is then a sudden change to a much more rapid increase in pressure drop. The length of the initial period of almost constant pressure varies with the type of plant and with the milk supply. For a tubular heat exchanger, Bell & Sanders (1944) found it to last for about 4 min (Fig. 2*a*). With a plate heat exchanger it can vary between 10 min and more than 40 min (Fig. 2*b*) (Burton, 1966*a*). The rapid rise in pressure after the first phase with a plate heat exchanger soon limits the length of run because of the risk of gasket breakdown, so that a consistent picture of the pressure drop during the second phase is rarely obtainable. In a tubular heat exchanger, where almost unlimited pressures can be applied, the pressure drop during the second phase increases linearly with time and quantity of milk processed.

If the milk has been preheld, a very different picture appears. In practice, the degree of preholding must be sufficient to give a useful improvement in deposition and yet be possible within a practicable continuous heat treatment process. Bell & Sanders (1944) suggested that the soluble proteins of milk were an important factor in deposit formation, and consequently that the effect of preholding arose from the denaturation of the soluble proteins before they reached the final heater. From

these considerations, a preholding treatment of 4–6 min at 85 °C, sufficient to denature most of the soluble protein, has been used widely.

After such preholding, the type A deposit is absent. The type B deposit occupies the whole of the final heating section, and now increases in amount through the section, being heaviest at the maximum temperature and extending also into the holding section. The composition of the deposit is similar throughout the section, with 12–20 % protein, 65–75 % ash, and 3–10 % fat. It is, therefore, comparable with the type B deposit found without preholding. However, it is curious that there is normally

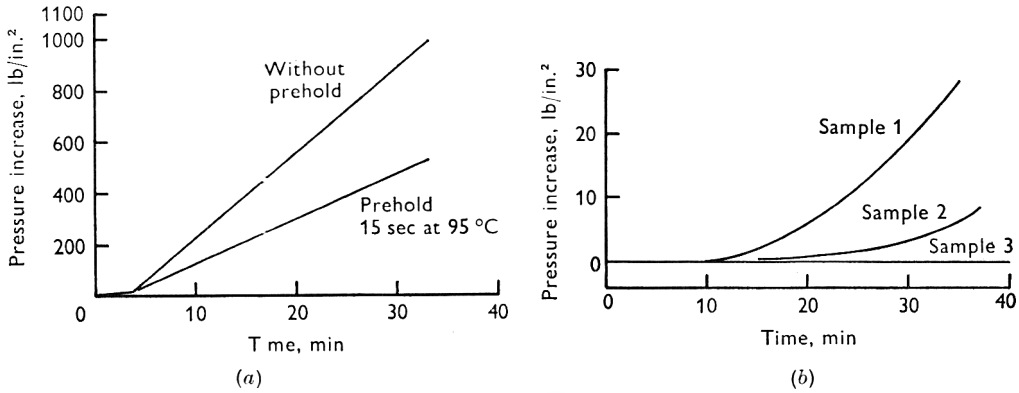

Fig. 2. Variation of pressure with time during operation of a UHT heat exchanger. (*a*) Tubular heat exchanger. (Derived from Bell & Sanders, 1944.) (*b*) Plate heat exchanger. (Derived from Burton, 1966*a*.)

much more of this deposit at the high temperature end of the heating section than if preholding is not used (cf. Figs. 1*a*, *b*). It is as if the precipitation of the type A deposit at an early stage removes some of the potential type B deposit which would otherwise be precipitated at a high temperature.

The type B deposit has been found to contain very small crystallites of β-$Ca_3(PO_4)_2$ with a distorted lattice. This compound has a Ca/P atom ratio of 1·5 : 1, but the ratio for the deposit has been found to be lower, about 1·3 : 1 : this may be attributed to the binding of cations other than calcium at the surface of the crystallites (Lyster, 1965). Ito & Nakanishi (1963*a*, *b*) agree that β-$Ca_3(PO_4)_2$ is an important mineral constituent.

Since the type A deposit, which is very effective in blocking flow passages, is absent after preholding has been used, there is much less increase in pressure during an operating run. This is shown in the curves of Fig. 2 (*a*) for a tubular-heat exchanger. Pressure is therefore much less of a factor in limiting the length of run. Temperature now becomes more important. The deposit is at its heaviest in the highest temperature regions of the heat exchanger, reducing the heat transfer coefficients at the last stage of heating where there is no chance for any later compensation if a set processing temperature is to be maintained. The control system can only react by increasing the temperature of the heating medium until it can go no higher, and the milk processing temperature can be held no longer.

Although we have concentrated on the final heating section in this account, deposits which are rarely significant in plant operation occur in other sections.

Deposits occur in the sections before the final heating section if the temperature is high enough (above about 70 °C). The deposits formed first at the lowest temperature may take the form of tufts of white deposit, similar in appearance to type A, attached to a filmy clear deposit which covers the whole heating surface. This clear film is unusual in that it has a relatively high ash content for a deposit formed at low temperature (53 % ash, 46 % protein, as compared with 25 % ash, 60 % protein for type A deposit (Lyster, 1965)). Ito & Nakanishi (1967*b*) have observed that a layer adjacent to the heating surface in low-temperature sections bears a structural resemblance to the high temperature deposit, and is mainly of calcium phosphate. This may refer to a similarity between the clear film and the type B deposit.

If a preholder has not been used, small amounts of clear or white deposits may be found in the cooling sections. These have a high protein content, 95 % for the clear deposit in the first section of the cooler, and 85–90 % for the white, stringy deposit found later (Lyster, 1965).

Deposits are also found on the unheated surfaces of the holding section, after mixing of the milk with steam in a direct-heating UHT system. An analysis of such a deposit has shown an unusually high fat content (34 %), with 37 % ash and 20 % protein. The Ca/P atom ratio was 1·44, which is near to the 1·5 for $Ca_3(PO_4)_2$ (R. L. J. Lyster, private communication).

It is therefore clear that there are many different forms of deposit, varying widely in composition. Only rarely is a deposit found which is almost entirely of one component. In general, all the major components of milk are found; fat, protein and mineral constituents. The fat content is usually relatively low, but the proportions of mineral and protein can vary widely according to the type of deposit, the site of its formation, and any pretreatment.

FACTORS AFFECTING DEPOSIT FORMATION

Plant operating conditions

Gynning *et al.* (1958) have shown that, at pasteurizing temperatures, milk flow velocity and temperature difference between heating medium and milk are both important. Increase in flow velocity reduces the rate of deposition, but the effect becomes less with increasing processing temperature and at a temperature of 90 °C they found no effect of velocity. A higher differential between milk and heating medium temperatures also promotes deposition, but this factor is less important than the chemical characteristics of the milk.

Air content

Gynning *et al.* (1958) found also that air content of the heated milk was of great importance. Using a laboratory plate pasteurizer operating at 85 °C, they found that the total amount of deposit formed was reduced by between 50 and 75 % if air was removed from the milk before processing. Ito & Nakanishi (1967*a*) have confirmed the effect of air in laboratory experiments. Indirect-heating UHT sterilizers have been made with de-aerators before the final heating stage (Buchwald, 1965), but there appears to be no claim that air removal reduces the deposit formation.

It is probable that air only encourages deposit formation if it separates as bubbles

on the heating surface, and that its effect will be eliminated if sufficient pressure is maintained on the milk at all times to prevent separation.

Acidity

It has long been recognized that milk of high acidity is likely to coagulate in a heat exchanger, so that it is only to be expected that reduction in the pH of milk would give a larger amount of deposit within a normal range where coagulation does not occur. This effect has been demonstrated by Gynning *et al.* (1958), Burton (1961, 1965), Ito *et al.* (1962*a*) and Ito & Nakanishi (1967*a*). The relationship between deposit formation and pH is non-linear, so that the effect of pH becomes greater as the pH falls, as shown in Fig. 3. There is some indication not only that developed acidity increases the deposit formation, but also that milk of a naturally low pH tends to give more deposit than does milk of a naturally high pH. This is shown in Fig. 4, but it is clear that other factors present in different milks are more important quantitatively than natural pH variations.

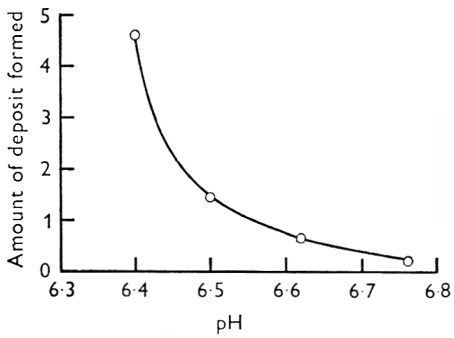

Fig. 3

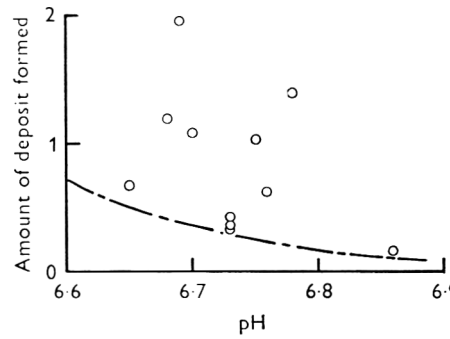

Fig. 4

Fig. 3. Variation of amount of deposit formed with pH, for the same milk with pH adjusted by addition of HCl. (Derived from Burton, 1965.)

Fig. 4. Variation of amount of deposit formed with pH, for commercial bulk raw milks. (Derived from Burton, 1965). —·—, curve from Fig. 3.

Age

Although developed acidity causes an increase in deposit formation, ageing of milk after production with no change of pH, either at normal temperature or under refrigeration, causes a marked decrease in the amount of deposit. After 24 h refrigerated storage, the amount of deposit formed may be only some 50 % of that when milk is fresh (Burton, 1964, 1966*a*). This effect is of some importance, as it runs counter to the general expectation that better results will be obtained with fresher milk. It has now been found advisable in practice not to use milk for UHT processing until it has aged for some hours under refrigeration (Ashton, 1966).

The amount of deposit falls approximately exponentially with time, as shown in Fig. 5 for both whole and separated milks (H. Burton, unpublished). The deposit is at its minimum after 15–30 h, and then appears to rise slightly. This rise may be due to the development of micro-organisms during storage, even under refrigeration. The reason for the fall in deposit formation during ageing is not yet understood. It may be

caused by redistribution of some of the mineral components following the drop in temperature when the milk leaves the udder, or it may be caused by the disappearance of some minor protein component on ageing (cf. Lindqvist & Storgårds, 1966).

Season

There are statistically significant variations in the build-up of pressure in a commercial UHT plant from season to season (Ito, Sato & Suzuki, 1963), and the weight of deposit obtained in a laboratory plate heat exchanger also varies with the season

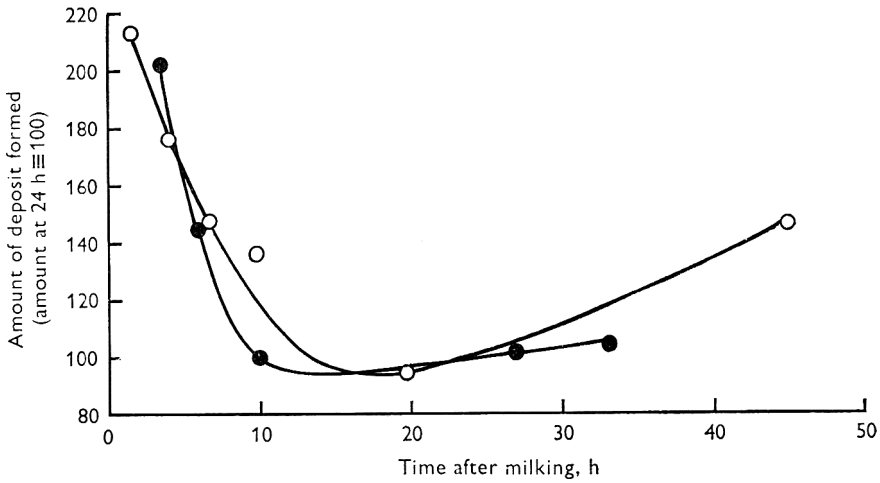

Fig. 5. Variation of amount of deposit formed with age of milk: ○——○, separated milk, ●——●, homogenized whole milk.

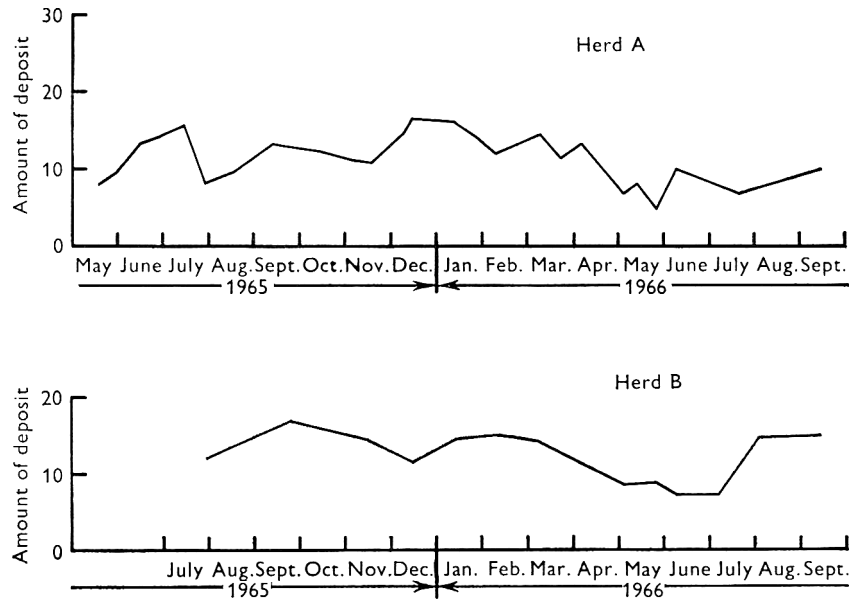

Fig. 6. Variation of deposit formation with season for the bulk milk of 2 herds. (From Burton, 1967.)

(Burton, 1966*a*). Using a laboratory method, the seasonal variation in the amount of deposit formed from the milk of single herds has been followed, with the results shown in Fig. 6 (Burton, 1967). For the herd milks examined, there is a minimum in late spring and early summer (May-July), with a broad maximum in the winter months from September to March. This variation is not merely a reflection of change in the total solids content of the milk with season, as the statistical correlation of deposit with total solids is poor. There is, however, a strong correlation ($P < 0{\cdot}01$) with fat content, so that the slight positive correlation of deposit with total solids results from the much more definite effect of fat.

These results were obtained with herds in the same locality, and it cannot be assumed that a similar seasonal variation will necessarily occur in every other region. Calving policy and feeding variations probably govern the form of the variation. For example, the low deposit period for herd B in 1966 coincided with a period when only grass was fed, while the increase in August coincided with the introduction of concentrates in the diet, and autumn calving.

Herd differences

There is little evidence on the amount and nature of differences between individual herds, although it is clear from the results for the 2 herds shown in Fig. 6 that there may be marked differences between herds only a few miles apart. It appears that during the experimental period the seasonal variation for herd B was much more uniform and less subject to rapid fluctuations than that for herd A. Herd B was a research herd, maintained on fixed diets for long periods. Herd A was a fully commercial one, and the short-term variations were probably caused by changes in feeding policy (Burton, 1967). As mentioned in the previous section, there were also indications that the longer-term variations in herd B were partly associated with feeding changes.

While such differences occur between neighbouring herds, there may obviously be large differences between bulk supplies from different areas, even supposing that the bulk supplies reach the processing dairy at a similar age and with similar bacteriological status. The variations which may be found between milks in practice are illustrated in Fig. 4. More studies are needed on this aspect of the problem. It is not known, for example, whether there are differences between the milks from different breeds of cows.

Preholding

As mentioned above, the value of preholding in reducing deposit formation was first shown by Bell & Sanders (1944). They suggested that the denaturation of the soluble proteins, associated with the removal of calcium and phosphorus from solution at high temperatures, was responsible for the effect of preholding. Protein denaturation is presumably responsible for the absence of the type A deposit after preholding (see above). However, a significant proportion of protein is present in the type B deposit even after preholding.

As judged by the rate of pressure change, Bell & Sanders (1944) found the effects of deposit formation to be reduced to half by preholding conditions of 95 °C for 15 sec, 74 °C for 10 min, or 71 °C for 30 min.

The effects of different preholding temperatures for a fixed holding time have

been studied using the hot platinum wire laboratory method (Burton, 1966*b*). The amount of deposit formed was found to vary linearly with the preholding temperature, as shown in Fig. 7.* A similar conclusion was reached by Ito & Nakanishi (1967*a*). This is not what one would expect if protein denaturation is the major effect of preholding: one would then expect a sudden effect as the temperature was raised to the point where denaturation occurred, with the effect complete within a comparatively small temperature range.

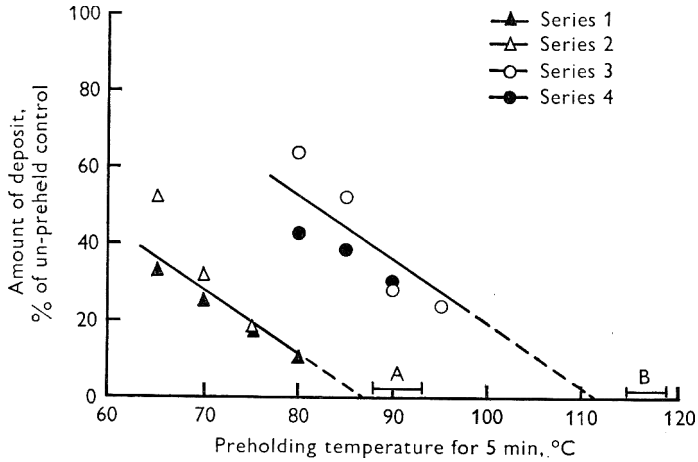

Fig. 7. Variation of deposit formation with preholding temperature. (A) Range of initial wire temperatures for series 1 and 2. (B) Range of initial wire temperatures for series 3 and 4. (Derived from Burton, 1966*b*.)

Furthermore, under different experimental conditions the effect of preholding was found to vary with the wire temperature. A higher temperature of the depositing surface required higher preholding temperatures to give the same reduction in the amount of deposit formed. It appeared that preholding became more and more effective as the preholding temperature approached the temperature of the depositing surface, and would have been completely effective only if preholding were carried out at the depositing temperature.

These results suggest that preholding is effective more because of changes in the mineral constituents of the milk than because of protein changes. A possible mechanism for the action of preholding is discussed later.

The value of preholding in reducing deposit formation is undisputed. It is unfortunate that a sufficient preholding, whether it acts through the minerals or the proteins, will denature much if not all of the soluble proteins and may have an adverse effect on the flavour of the milk after processing.

Electrical or magnetic treatment

Two possible types of treatment have been proposed. In the first, the milk is subjected to a small direct or alternating electric current before it reaches the heat

* These results are slightly different from those given in Burton (1966*b*), as it is now known that the hot wire results should be interpreted slightly differently to give a true measure of deposit formation in practice. The conclusions from the results are not changed.

exchanger, or it is passed through a strong magnetic field which produces similar currents by induction. In the second, the heating surface is made one pole of an electric circuit so that local changes are caused at the surface.

The first method has been claimed to reduce scale in boilers and several proprietary units are available for feed water treatment (Anders, 1963). One such unit, the Hydrotron, has been claimed to reduce deposit formation in an HTST pasteurizer (Leeder & Martin, 1952; Leeder, 1956), but more recently a comprehensive study (Mhatre, 1962) has failed to show any effect on either the composition or amount of deposit. Practical tests in this country have not been successful.

The second method has been found useful in preventing cheese curd adhesion to the vat surface. In a heat exchanger the amount and structure of the deposit can be modified, but it cannot be removed entirely, and there are compensating disadvantages (Burton, 1959). In view of the difficulties of applying the method to heat exchangers, it is unlikely to be useful in practice.

POSSIBLE MECHANISMS OF DEPOSIT FORMATION

It seems that deposit formation can be considered as 2 separate processes. First, high temperature produces a condition in the milk in which some of the milk solids are no longer in true solution but are in such a state that they will either adsorb to a surface or aggregate. Then secondly, if a surface is present the solids which have left true solution will adsorb to it and extend to form a deposit. If a surface is not present the solids will aggregate as an alternative, and will then be no longer available to form a deposit later at a surface.

Mineral salts and the effects of temperature near a heating surface

It is comparatively simple to build up a qualitative picture of deposit formation on the heating surface of a heat exchanger, and hence to explain some of the factors which have been found to influence deposit formation. The picture is given some precision if it is assumed that the reduction in the solubility of the milk salts at high temperatures forms the first stage, and the second stage involves the slow formation of crystal nuclei and the growth of the nuclei into crystals which constitute the deposit.

In Fig. 8(*a*), θ_1 is the temperature of the heating surface (this will be lower than the temperature of the heating medium, but for a constant heat flow it will itself be constant), and θ_2 is the temperature of the liquid that is flowing past the surface and being heated by it. If a liquid flows in turbulent motion, as in a heat exchanger, parallel to a solid surface, the longitudinal velocity increases rapidly from zero at the wall to an almost constant value a short distance away. This velocity gradient is accompanied by a corresponding sharp change in liquid temperature, from θ_1 at the surface to θ_2 within the body of the liquid as shown. Normally, the situation is treated as if an insulating stationary layer of thickness δ covers the surface, so that a linear fall in temperature from θ_1 to θ_2 occurs within the layer.

The liquid temperature is at its highest, θ_1, at the heating surface and will there have its highest level of supersaturation with respect to the mineral salts, e.g. $Ca_3(PO_4)_2$. Under supersaturated conditions, in time crystal nuclei will form and

grow. The surface will encourage the formation of nuclei, and crystal growth will then occur to an amount depending on the degree of supersaturation and the volume of supersaturated solution. It can be shown that, in these circumstances, there will be a linear increase in deposit thickness with time, if the rate of heat flow is constant (Reitzer, 1964).

This will eventually lead to a condition as in Fig. 8(*b*). Because of the insulating effect of the deposit layer, the heating surface temperature θ_1 increases, and the deposit surface temperature θ_s takes its place. The inner layer of deposit is overheated by the rise in temperature of the heating surface. The thickness of the stationary layer may not be the same as with a clean heating surface because of the roughness of the deposit surface which is now supplying heat to the milk.

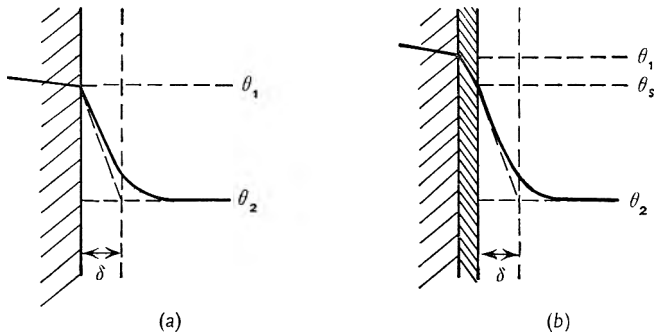

Fig. 8. Temperature conditions at a heating surface: (*a*) without deposit; (*b*) with deposit.

If the heating medium to milk temperature differential is increased, θ_1 is increased for the same θ_2. The degree of supersaturation at the surface will increase, and there will be more rapid nucleation and more rapid subsequent crystal development. This is in accordance with the findings that an increased differential gives more deposit.

If the velocity of the milk is increased, for hydrodynamic reasons the thickness of the stationary layer δ will decrease. θ_1 will be lowered slightly for the same θ_2 because of the lower thermal resistance of the stationary layer, so the differential temperature is reduced. The volume of milk heated to temperatures approaching θ_1 will also be less so that the total amount of material crystallizing will be less. Increase in milk velocity therefore tends to decrease the amount of deposit, but cannot eliminate it entirely because some of the milk still reaches temperature θ_1. This again is in accordance with experience.

The non-linear increase in pressure drop across an indirect heat exchanger has been mentioned earlier (see Figs. 2(*a*), (*b*)). A delay before a marked increase in pressure occurs is widespread in applications where scale forms on a heating surface. Reitzer (1964) suggests that it arises where there is only a slight degree of supersaturation at the heating surface and the spontaneous nucleation necessary for crystal growth is rare. After the slow formation of sufficient nuclei, deposit formation is governed by the rate of supply of new material to continue crystal growth.

The effect of preholding

The observed effects of preholding can be similarly explained on the basis of supersaturation and crystallization. At the preholding temperature, the mineral

salts will be supersaturated to a degree depending on the temperature. If a surface is available, nuclei will form near the surface and deposition will occur; this corresponds to the conditions at the inlet of a preholding vessel. Where a surface is not available, as in the main volume of a preholding vessel, spontaneous nucleation will take place in the milk itself, and crystallization will occur to remove the supersaturation. This material will remain as a component of the milk, and will be no longer available to form deposit later on any surface. Such nucleation as occurs on the walls of a preholding vessel will give rise only to limited crystal growth because firstly, in the low-velocity conditions in a preholding vessel, it will be able to draw on only a limited volume of supersaturated milk, and secondly, the walls of the vessel are likely to be relatively cool so that the degree of supersaturation near to them will be less than elsewhere. No deposit therefore occurs on the walls of a preholding vessel.

After preholding, further deposit will only form when the temperature has been raised above that of preholding to give sufficient degree of supersaturation for a further stage of nucleation and crystallization. The effectiveness of preholding will depend on the temperature, and can only be complete if the preholding temperature is near to the final processing temperature.

The involvement of proteins and fat

Although this hypothesis is concerned only with the mineral salts of milk, proteins must be involved in some way in deposit formation. Proteins seem always to be present as a component of deposit, even when the major constituent is ash, and they normally appear to be denatured or even degraded into non-protein compounds (Ito & Nakanishi, 1964; Nakanishi & Ito, 1966). Prior denaturation of soluble proteins during preholding results in marked changes in the structure and composition of the deposit formed on plant surfaces, as has been described.

Lyster (1966) has found evidence for an interaction between denatured β-lactoglobulin and precipitated calcium phosphate when a solution of milk salts and β-lactoglobulin is heated to 100 °C. This seems likely to explain, at least in part, the intimate relation between minerals and protein in the deposits from heated milk.

It does not follow that the presence of protein necessarily increases the amount of deposit. Burton and Lyster found that the addition of β-lactoglobulin appeared to depress the amount of deposit formed, both in milk (Burton, 1964) and in artificial milk salt systems (Lyster, 1966). On the other hand, Ito & Nakanishi (1967*a*) found that, when deposits from combinations of the 3 separate constituents tricalcium phosphate, casein and whey powder were compared, tricalcium phosphate + whey powder gave the largest amount of deposit.

Although deposit formation is highly correlated with fat content (Burton, 1967), it is unlikely that the fat itself is closely involved, since the amount of deposit does not vary linearly with fat content, and since it is well known that the deposit problem exists with separated milk as well as with whole milk. It is more likely that deposition is influenced by some minor milk constituent which is closely associated with the fat and varies with it, but which is also present in separated milk. A phospholipid would be such a component, as phospholipids have been found to vary seasonally in amount in a way similar to that found for deposit formation (Holden, Aceto, Dellamonica & Calhoun, 1966). Ito and his co-workers (Ito *et al.* 1963; Ito & Nakanishi, 1967*a*) have

found the amount of deposit to be correlated with physical properties of the milk, e.g. surface tension, which might be affected by a surface-active compound such as a phospholipid, but Ito & Nakanishi (1966*c*) were unable to detect any preferential inclusion of phospholipids in deposits.

This hypothetical minor component may affect deposit formation by controlling the availability of nuclei for crystal growth: the differences between milks seem to lie in practice in the length of the initial period when the operating pressure in a heat exchanger remains constant (cf. Fig. 2(*b*)), perhaps, as Reitzer (1964) suggests, because the availability of nuclei is limiting, rather than in the rate of increase of pressure later when the controlling factor is the amount of supersaturated material to continue crystal growth.

Deposits in directly heated systems

The mechanisms suggested so far are not confined only to indirectly heated systems. Whatever the system of heating, if mineral salts become supersaturated, nuclei will form and crystal growth will occur. In a directly heated system, a large quantity of precipitable material becomes available immediately after mixing of the milk with steam. Some of this material is carried to the enclosing surfaces by turbulence, and deposition will then occur as if the surfaces were responsible for the heating. Deposit occurs throughout the whole of the heating system, but it decreases from the mixing point to the expansion valve where cooling occurs. It is noteworthy that the pressures in the vicinity of a steam injector heating milk to 140 °C have been found to vary non-linearly with time in the same way as the pressures in an indirect heater (cf. Fig. 2(*a*), (*b*),) suggesting a similar mechanism of deposit formation (A. G. Perkin & H. Burton, unpublished).

Sediment and deposit

Where supersaturated material crystallizes and forms aggregates in the body of the milk without deposition, the aggregates may subsequently appear as sediment in the UHT milk after storage or centrifugation. This sediment presents a technological problem (Samuelsson, Gynning & Olsson, 1962), and has been found to be very serious in some areas, such as Southern Italy and South Africa, at times of poor pasture. One might expect the amount of sediment in stored processed milk to be less after an indirectly heated process than after a directly heated process, since in the former a greater proportion of the crystallized material will form a deposit in the plant and less will remain in the milk. Evidence on this is conflicting: Samuelsson & Holm (1966) found more sediment with indirectly heated milk, but Corradini, Dellaglio & Bottazzi (1967) found the reverse.

The effect of deposit–surface interaction

In the main, the precipitated material becomes troublesome only when it adheres to a surface to form a deposit, so that an important factor is the adhesion of the material to the surface. The first stage of adhesion is presumably one of adsorption of deposit to surface, followed by an addition of deposit to deposit. It has been shown (see Jennings, 1965) that there are probably 2 types of milk soil present on a surface, one which is relatively easy to remove and one which is difficult to remove. The latter may well be adsorbed material, while the former is material which is attached to soil

rather than to solid surface. This points to the possibility of reducing deposit formation by interfering with the adsorption stage, e.g. by saturating adsorption sites before processing begins or by otherwise preventing the initial adhesion. Unfortunately there is no information on this possibility and no evidence that it might be practicable. Even such a material as polytetrafluoroethylene (PTFE), which has very poor adhesion characteristics, will build up substantial deposits in heated milk. This suggests that the growth of deposit on deposit is sufficiently easy and rapid for a mechanically stable structure to develop even when the initial deposit-surface adhesion is poor.

CONCLUSIONS

There is still considerable scope for research into deposit formation and its many associated problems which range from the interactions of the different components of the milk to the processes of adsorption to surfaces. Much further knowledge is required before alternatives to preholding can be put forward for limiting deposit formation in practical heat treatment plant. It is hoped that some of the speculations in this discussion will promote further work.

I am indebted to Dr R. L. J. Lyster for many stimulating discussions on this subject over a long period.

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Tables should be numbered and should carry headings describing their content. They should be comprehensible without reference to the text. They should be typed on separate sheets and their approximate positions in the text indicated.

ILLUSTRATIONS

Line drawings, which must be originals, should be numbered as Figures and photographs as Plates, in Arabic numerals. Drawings should be in indian ink, on Bristol board or cartridge paper. However, a technique which may be more convenient to authors is to use a double-sized piece of tracing paper, or translucent graph paper faintly lined in *blue* or *grey*, folded down the centre with the drawing on one half and the other half acting as a flyleaf.

Attached to every figure and plate there should be a translucent flyleaf cover on the outside of which should be written legibly: (*a*) title of paper and name of author; (*b*) figure or plate number and explanatory legend; (*c*) the figures and lettering, which are intended to appear on the finished block, in the correct positions relative to the drawing underneath. For each paper there should be also a separate typed sheet listing figure and plate numbers with their legends, and the approximate positions of illustrations should be indicated in the text.

As a rule the photographs and diagrams should be about twice the size of the finished block and not larger over-all than the sheets on which the paper itself is typed. For general guidance in preparing diagrams, it is suggested that for a figure measuring 9 in. × 6 in. all lines, axes and curves, should have a thickness of 0·4 mm, thus ————, Graph symbols in order of preference should be ○ ●, △ ▲, □ ■, × +, and for a 9 in. × 6 in. graph the open circles should be $\frac{1}{8}$ in. in diam. The open triangles should be large enough to contain circles of $\frac{3}{32}$ in. diam. and the open square circles of $\frac{1}{8}$ in. diam. The crosses should have lines $\frac{1}{8}$ in. long. The block symbols should be slightly smaller than the corresponding open symbols. Scale marks on the axes should be on the inner side of each axis and should be $\frac{1}{8}$ in. long.

REFERENCES

In the text, references should be quoted by whichever of the following ways is appropriate: Arnold & Barnard (1900); Arnold & Barnard (1900*a*); Arnold & Barnard (1900*a*, *b*); (Arnold & Barnard, 1900). Where there are more than 2 authors all the surnames should be quoted at the first mention, but in subsequent citations only the first surname should be given thus, Brown *et al.* (1901). If there are 6 or more names *et al.* should be used in first instance. Also, if the combinations of names are similar, e.g. Brown, Smith & Allen (1954); Brown, Allen & Smith (1954), the names should be repeated each time. Reference to anonymous sources is not acceptable.

References should be listed alphabetically at the end of the paper, titles of journals being abbreviated as in the *World List of Scientific Periodicals*. Authors' initials should be included, and each reference should be punctuated in the typescript thus: Arnold, T. B., Barnard, R. N. & Compound, P. J. (1900). *J. Dairy Res.* **18**, 158. References to books should include names of authors, names of editors, year of publication, title, town of publication and name of publisher in that order, thus, Arnold, T. B. (1900). *Dairying*. London: Brown and Chester.

It is the duty of the author *to check all references* and to ensure that the correct abbreviations are used.

SYMBOLS AND ABBREVIATIONS

The symbols and abbreviations used are those of British Standard 1991: Part 1: 1954, *Letter Symbols, Signs and Abbreviations*.

DESCRIPTIONS OF SOLUTIONS

Normality and molarity should be indicated thus: N-HCl, 0·1 M-NaH_2PO_4. The term '%' means g/100 g solution. For ml/100 ml solution the term '% (v/v)' should be used and for g/100 ml solution the correct abbreviation is '% (w/v)'.

REPRINTS

Order forms giving quotations for reprints are sent to authors with their proofs.

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