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L. A. MABBITT   and   **J. A. B. SMITH**

B.Sc., Ph.D.   C.B.E., Ph.D., D.Sc., F.R.I.C.

The National Institute for Research in Dairying,   The Hannah Dairy Research Institute,

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CORRECTION

The separation of milk protein on dextran gel

R. D. HILL AND RAIONE R. HANSEN

For lactalbumin and lactoglobulin *substitute* α-lactalbumin and β-lactoglobulin, respectively, throughout the paper.

The effect on growth rate and on milk yield and composition of finely grinding the hay and cooking (flaking) the maize in mixed diets for growing and for milking heifers

By C. C. BALCH, W. H. BROSTER, J. A. F. ROOK
AND VALERIE J. TUCK

National Institute for Research in Dairying, Shinfield, Reading

SUMMARY. In experiments with 20 growing and 24 milking heifers, receiving mixed diets of hay and concentrates, the effects of grinding the hay and cooking (flaking) the maize constituent of the concentrates were examined.

The treatments had no effect on milk yield, solids-not-fat content of the fat-free milk serum, or liveweight changes in the milking cows. In contrast the milk fat content was very significantly reduced when ground hay was given, and the depression was significantly greater with the concentrates containing flaked maize than with those containing maize meal.

The rate at which yearling heifers grew was not influenced by the treatments. A small subsidiary experiment suggested that this equal growth was achieved in spite of substantial depression in the digestibility of diets containing ground hay.

The volatile fatty acids produced during fermentation in the rumen have been shown to vary in their nutritive properties. Armstrong & Blaxter (1957) found that for lipogenesis in the adult sheep the value of the acids increases with increasing chain length. Rook & Balch (1961) showed that the individual acids have characteristically different effects on the synthesis of the various constituents of milk. In consequence the nature of the end products of fermentation must be one factor affecting the utilization of foods for productive purposes.

It is well known (see Rook, 1961) that the proportions of the volatile fatty acids produced in the rumen from mixed diets can be changed by altering the physical condition of the diet. Grinding the hay and cooking (flaking) the maize portion of the concentrate have frequently been found to reduce the proportion of acetic acid and to increase the proportion of propionic acid in the total volatile fatty acids. Three experiments have, therefore, been conducted, using mixed diets of hay and concentrates to determine the effects of grinding hay and cooking concentrates on the utilization of the diet for growth and for milk production.

Foods

The same hay and concentrate mixtures were used in each experiment.

The hay, composed mainly of Italian rye-grass (*Lolium italicum*) was cut on 16 June 1960 from one uniform field in Lincolnshire. It was harvested under good conditions and stored in 2 ricks. The hay was baled from the ricks and was offered to the animals either in the long state or after fine grinding. For grinding, half the hay from each rick was chopped and passed through a grass drier to remove most of the moisture. A detailed analysis of the particle size of the ground hay was given by Campling, Freer & Balch (1963).

Two mixtures of concentrates (mixtures *X* and *Y*) were used; large batches of these were prepared as required with the ingredients listed in Table 1. The mixtures differed only in that mixture *X* contained 46% flaked maize and mixture *Y* contained 46% maize meal. The maize was purchased from commercial sources, care being taken to ensure that the maize meal was prepared from the same shipment of American maize as that used for flaking. Flaked maize is prepared by soaking maize in water for 24 h or longer, heating the mixture with steam to 200 °F and then passing it between rollers. The flaked maize was ground before inclusion in mixture *X*.

Table 1. *Constituents of concentrate mixtures* (%)

Constituent	Mixture *X**	Mixture *Y**
Flaked maize	46	—
Maize meal	—	46
Wheat offals	7	7
Barley	7	7
Decorticated groundnut meal	20	20
Copra cake	5	5
Palm kernel cake	5	5
Molasses	7	7
Salt	1	1
Calcium carbonate	1	1
Dicalcium phosphate	1	1

* A vitamin concentrate was added to each mixture in amounts supplying 5,000,000 i.u. vitamin A and 1,000,000 i.u. vitamin D per ton.

Diets

The 6 diets contained 2 proportions of hay and concentrates, as shown in Table 2. For diets *C*, *D* and *F* the ground hay and concentrates were pelleted together; for diets *A*, *B* and *E* the concentrates were pelleted, but the hay was given in the long form. During pelleting the temperature of diets containing ground hay rose to 140 °F and of concentrates not containing hay to 120 °F.

Allowances of food were weighed daily for each animal and were given in 2 equal meals, the milking cows receiving their food before milking.

Representative samples of the foods offered were taken throughout the experimental periods and, together with samples of the faeces collected in the digestibility trial, were analysed by standard procedures. The mean composition of the foods is given in Table 3.

Expt. 1. *Milking cows*

Twenty Friesian heifers from the Institute herd were placed on experiment 10 weeks after calving in their first lactation. They were blocked according to milk yield, date of calving and pre-partum level of feeding. The animals in each block were allocated at random to an experimental design in which they received diets *A–D*, according to a 4 × 4 Latin square balanced for residual effects (Lucas, 1956) in periods of 5 weeks.

Table 2. *Content of hay and concentrates in the diets* (%)

Diet	Long hay	Ground hay	Concentrate X containing flaked maize	Concentrate Y containing maize meal
A	40	—	60	—
B	40	—	—	60
C*	—	40	60	—
D*	—	40	—	60
E	60	—	—	40
F*	—	60	40	—

* Diets *C, D* and *F* were given to the animals as a mixture of ground hay and concentrates pelleted together.

Table 3. *Mean composition of the foods and diets*

Food or diet	Dry matter, %	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Ash
				% of dry matter		
Long hay	84·9	8·7	1·4	32·9	50·0	7·1
Ground hay	92·8	8·2	1·6	30·5	51·7	8·0
Concentrate X	86·7	20·2	3·1	6·8	63·4	6·5
Concentrate Y	87·6	20·2	3·4	6·5	63·4	6·6
Diet A*	86·0	15·6	2·4	17·2	58·0	6·7
Diet B*	86·5	15·6	2·6	17·1	58·0	6·8
Diet C	89·1	15·1	2·6	15·0	60·1	7·0
Diet D	89·8	15·3	2·8	16·7	57·7	7·6
Diet E*	86·0	13·3	2·2	22·3	55·4	6·9
Diet F	92·0	12·1	2·3	19·9	58·0	7·7

* Dietary composition calculated from the composition of the hay and concentrates.

The amounts of the diets offered in the first week of the experiment were calculated to provide 105% of the energy allowance recommended by Woodman (1957) for cows of this weight and yield. For the purposes of this calculation it was assumed that the mixture of 40% hay and 60% concentrates had a starch equivalent of 55. The daily diet was reduced by 1 lb every 5 weeks to compensate for decline in milk yield.

The cows were fed and milked twice daily in a conventional cowshed with partitions between each cow to prevent stealing; at night they were housed in yards with wood shavings for bedding. Yields were recorded at each milking. Samples from 6 successive milkings per week were bulked for estimation of milk fat and solids-not-fat.

Expt. 2. Growing heifers

Twenty-four heifers from the Institute herd averaging about 1 year of age and 450–600 lb in weight, were used for the experiment. They consisted of 12 Friesians, 4 Ayrshire and 8 Channel Island cattle; these groups included 8 pairs of monozygous twins. From grazing the heifers were brought into the experimental pens where they received a diet of hay and concentrates for 31 days before the experiment began.

The heifers were allocated to blocks of four according to breed and liveweight, with the restriction that members of twin pairs went to the same block; twin pairs were then both allocated to the same level of feeding, treatments involving the experimental preparation of the food being allocated within 2 pairs.

The experiment was divided into 2 parts. In part 1 a comparison was made of diets *E* and *F*, each containing 60% hay and 40% concentrates, and given in amounts, which will be called 'low' or 'high', adjusted for daily rates of gain of about 1·0 or 1·6 lb. To achieve these gains the daily allowances for each 2 animals in each block receiving the same level of diets *E* and *F* were adjusted every time the animals were weighed, according to the amount the mean liveweight for the 2 animals deviated from the desired gain. This system proved easy to operate. The diets were given for 81 days.

In part 2 of the experiment diets *B* and *D*, each containing 40% hay and 60% concentrates, were compared at higher rates of gain during a period of 75 days. The diets were offered to 1 pair of heifers in each block at 'low' rates, sufficient to give 1·6 lb daily gain, and to the other pair at 'high' rates equal to the *ad lib.* intake of the diet consumed in least amounts. Rations were fixed at the beginning of the period. Animals were re-allocated to treatments so that individuals received the same food preparation as in part 1, but levels of feeding were re-randomized factorially within pairs of the original block.

The procedures for feeding and weighing the animals were as described by Bailey & Broster (1957), the animals being bedded on wood shavings. Care was taken to mix new shavings with old bedding and this largely prevented the heifers from eating their bedding.

Throughout the experiment a system of equalized feeding was followed whereby if an animal left food uneaten, an equal amount was deducted on the following day from the allowance of the other animal in the same block receiving the same level of feeding.

Expt. 3. Digestibility trials

The digestibilities of diets *B*, *D*, *E* and *F* were determined in trials with 2 dry cows. Supplies of the other diets were not sufficient to permit a determination of digestibility. The diets were given in amounts sufficient to supply 24 lb dry matter with diets *E* and *F* and 17 lb dry matter with diets *B* and *D*. The cows received each diet for 14 days before the start of a 10-day collection period. Faeces were collected by means of the arrangements described by Balch, Johnson & Machin (1962).

Expt. 1. *Milking cows*

Three heifers occasionally had mild bloat when receiving diets containing ground hay, but treatment was not necessary. Apart from this the animals remained in good health.

Table 4. *Expt.* 1. *Effects of type of hay and preparation of maize in the concentrate mixture on the yield and composition of milk*

(Values are means per day for 20 cows in the last 2 weeks of 5-week periods.)

Diet	Type of hay	Type of maize	Food intake,* lb	Milk† yield, lb	Milk fat content		Milk solids-not-fat		Solids-not-fat of fat-free serum, %
					%	lb	%	lb	
A	Long	Flaked	25·3	26·4	3·60	0·95	8·69	2·29	9·01
B	Long	Meal	25·3	26·6	3·60	0·96	8·70	2·32	9·02
C	Ground	Flaked	24·1	25·7	2·42	0·62	8·80	2·26	9·02
D	Ground	Meal	24·6	25·6	2·77	0·71	8·76	2·23	9·01

* The amounts of hay offered were adjusted to allow for the higher dry matter content of the ground hay. The values presented here have been calculated from the dry matter intake on the assumption that the dry matter content of the hay was the same as that of the ground hay.

† Mean values based on milk yields during 3-day sampling period in each week, see p. 3.
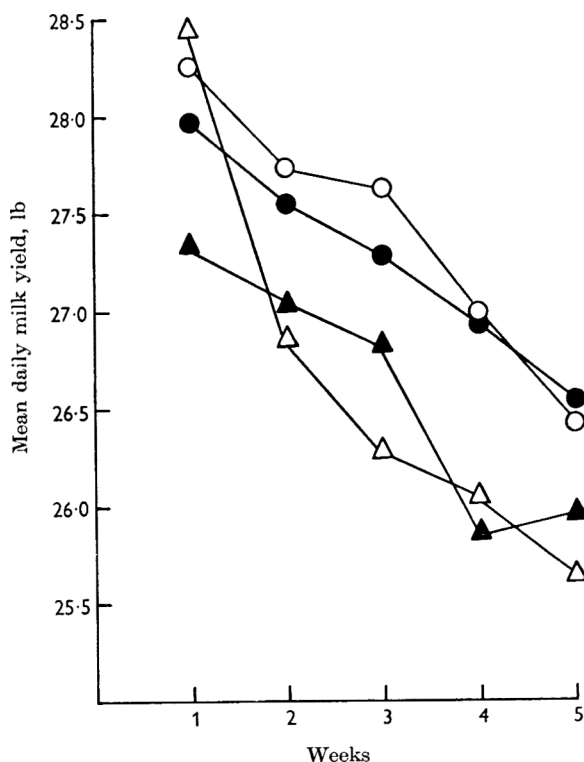
Fig. 1. Expt. 1. Mean daily milk yield of 20 cows receiving 4 diets: ○, long hay and concentrates containing flaked maize; ●, long hay and concentrates containing maize meal; △, ground hay and concentrates containing flaked maize; ▲, ground hay and concentrates containing maize meal.

The mean intake of each diet and mean values for the yield and composition of milk in the last 2 weeks of each experimental period are given in Table 4. Weekly mean values for the yield and composition of the milk are plotted in Figs. 1, 2 and 3.

There was no refusal of diets A and B which contained long hay. There were, however, refusals of diets C and D containing ground hay, the mean daily refusals being 1·4 and 0·7 lb/cow, respectively. Refusals varied markedly between animals, the maximum daily refusals in any 1 week being 11·7 and 8·4 lb/cow, respectively. Refusals of diet C decreased appreciably with time; refusals of diet D were more regular over the experimental period. The mean daily refusals for weeks 4 and 5, for which period milk production data are given in Table 4, were 1·2 and 0·7 lb/cow for diets C and D, respectively.
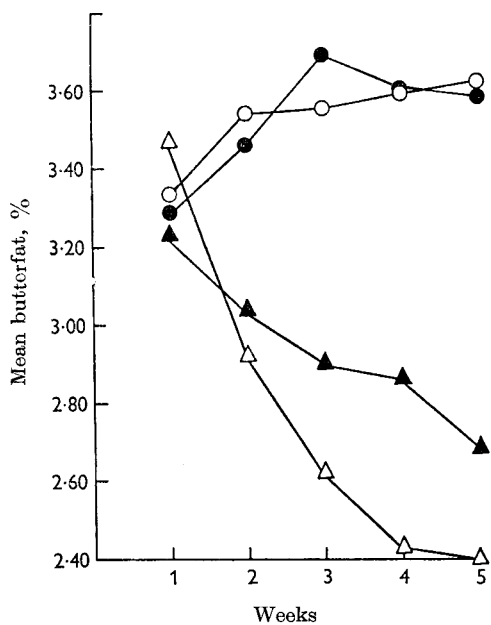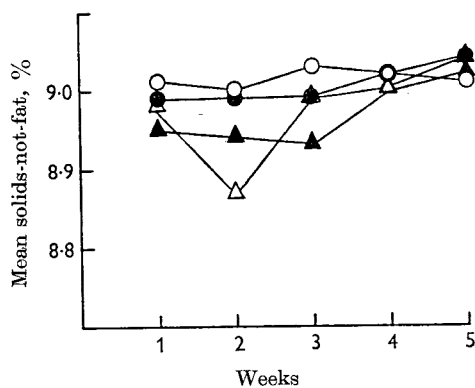
Fig. 2   Fig. 3

Fig. 2. Expt. 2. Mean fat content in milk from 20 cows receiving 4 diets: ○, long hay and concentrates containing flaked maize; ●, long hay and concentrates containing maize meal; △, ground hay and concentrates containing flaked maize; ▲, ground hay and concentrates containing maize meal.

Fig. 3. Expt. 2. Mean solids-not-fat content in fat-free milk serum from 20 cows receiving 4 diets: ○, long hay and concentrates containing flaked maize; ●, long hay and concentrates containing maize meal; △, ground hay and concentrates containing flaked maize; ▲, ground hay and concentrates containing maize meal.

The mean value for milk fat content with both diets containing long hay was 3·60 %. With diet C, containing ground hay and flaked maize, and with diet D, containing ground hay and maize meal, the fat content was significantly ($P < 0.001$) depressed to mean values of 2·42 and 2·77 %, respectively. The difference of 0·35 % between the mean values for treatments C and D was significant ($P < 0.05$). The extent of the depression in fat content varied markedly from cow to cow. Only one animal showed no response in milk fat percentage to either treatment C or D. All other animals responded to both treatments. With treatment C, three animals showed

declines of less than 0·3 %, three of 0·3–1·0 %, nine of 1·0–2·0 % and four of greater than 2·0 %.

No significant differences were found between the treatment mean values for milk yield and the solids-not-fat content of the fat-free serum. Animals that refused food when diets *C* and *D* were given invariably showed a loss in milk yield during the period of food refusal, but with the recovery of appetite milk yield also recovered. There was no obvious relationship between the amount of diet refused and the extent of the depression in milk fat content.
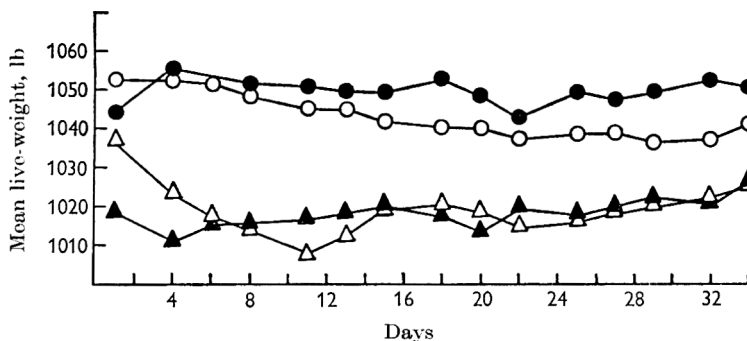
Fig. 4. Mean liveweight of 20 cows receiving 4 diets: ○, long hay and concentrates containing flaked maize; ●, long hay and concentrates containing maize meal; △, ground hay and concentrates containing flaked maize; ▲, ground hay and concentrates containing maize meal.

During the first week on diets containing ground hay, liveweights fell sharply (Fig. 4), due most probably to a reduction in the weight of gut contents. A tendency for the weight of the animals to decrease with diet *A* and to increase with diets *C* and *D* was too slight to be meaningful.

Expt. 2. Growing heifers

The food intakes of the animals in expt. 2 are summarized in Table 5 and the mean rates of gain in liveweight are given in Table 6.

The values in Table 5 show that closely similar intakes of dry matter/100 lb liveweight were achieved in animals receiving the same level of feeding. Some heifers, receiving the high level of diet *E*, left small amounts of long hay uneaten in the early stages of part 1. Also, with the increase in the proportion of concentrates in the diet, heifers receiving diet *C ad lib.* in part 2 consumed less than those receiving diet *F* at the high, but controlled, level in part 1. One heifer receiving, in part 2, the low level of diet *C* containing ground hay, left so much uneaten that the pair of which she was a member was removed from the experiment. In general, portions of diets containing ground hay were left uneaten much more frequently than those containing long hay.

One heifer receiving diet *F* at the low level in part 1 developed severe bloat on the 37th day. In spite of treatment with trochar and cannula the disorder recurred several times, eventually leading to removal of the animal and her mate from the experiment. Only one animal showed mild bloat in part 2.

Part 1. Immediately the change to the experimental diets was completed, marked falls in liveweight were observed in heifers receiving the low level of feeding with

diets E and F and the high level of feeding with diet F. These falls were evidently due to reductions in gut contents, and to allow for this change the mean rates of live-weight gain (Table 6) were calculated from regression coefficients based on weighings made during the 9th–72nd day of full experimental treatment.

Table 5. *Expt. 2. Mean daily intake of foods (lb) by 24 growing heifers*

Part of expt.	Diet	Level of feeding	Hay	Concentrates*	Mixed† diet	Dry matter/ 100 lb. initial liveweight
1‡	E	High	11·3	6·4	—	2·46
		Low	8·2	4·6	—	1·96
	F	High	—	—	16·4	2·54
		Low	—	—	12·0	2·00
2§	B	High	7·1	9·2	—	2·20
		Low	6·7	8·5	—	2·00
	C	High	—	—	15·4	2·16
		Low	—	—	14·2	1·96

 * Concentrates given with long hay contained maize meal, concentrates given with ground hay contained flaked maize.
 † The mixed diet consisted of ground hay and concentrates pelleted together.
 ‡ In part 1 all diets consisted of 60 % hay, or ground hay, and 40 % concentrates.
 § In part 2 all diets consisted of 40 % hay, or ground hay, and 60 % concentrates.

Table 6. *Expt. 2. Effect of grinding hay and preparation of maize in the concentrate mixture on the rate of gain in liveweight of 20 growing Friesian heifers receiving diets of hay and concentrates*

(Values are means from the last 9 weeks of each period.)

Part of expt.	Diet*	Level† of feeding	Initial liveweight, lb	Liveweight at beginning of period used to calculate liveweight gain, lb	Rate of gain in liveweight, lb/day
1	E	High	571	590	1·43
		Low	547	544	0·89
	F‡	High	575	571	1·46
		Low	557	545	0·98
2	B	High	619	632	1·61
		Low	665	659	1·15
	C§	High	617	639	1·42
		Low	661	670	1·18

 * Details of the diets are given in Table 2, six animals received each diet.
 † Details of the level of feeding are given on p. 4.
 ‡ One pair of heifers receiving treatment F was discarded, see p. 7.
 § One pair of heifers receiving treatment C was discarded, see p. 7.

The mean daily rates of gain (Table 6) were 1·44 lb with the high level of feeding and 0·93 lb with the low level of feeding, the difference between these being highly significant ($P < 0·01$); these were reasonable approximations to the intended gains of 1·6 and 1·0 lb, respectively. The mean daily rate of gain with treatment E was 1·16 lb and with treatment F 1·22 lb; the difference was not significant ($P > 0·05$),

neither were the differences between the treatments significant at either the high or low level of feeding.

Part 2. The mean daily rates of gain obtained of 1·52 lb with the high and 1·16 lb with the low level of feeding were less than had been intended, but the difference in rate of gain was significant ($P < 0·01$). The heifers receiving the 2 levels of diet B, containing long hay, grew at 1·38 lb/day compared to 1·30 lb/day with the 2 levels of diet C, containing ground hay; these differences were not significant ($P > 0·05$).

Expt. 3. Digestibility trial

The results of the digestibility trial are given in Table 7.

With diets E and F, containing 60 % hay and 40 % concentrates, grinding the hay and replacing the maize meal by flaked maize, reduced the digestibility of the dietary dry matter from 66·4 to 53·5 % and of the organic matter from 67·9 to 56·6 %. This depression was evidently due mainly to a depression of the digestibility of crude fibre from 52·9 to 15·4 %.

Table 7. *Expt. 3. Mean digestibility* (%) *of diets B, D, E and F in 2 dry cows*

Diet*	Type of hay	Type of maize	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Ash
B	Long	Meal	71·3	73·8	70·7	36·3	54·2	80·3	37·1
D	Ground	Meal	68·2	70·2	77·6	75·9	32·3	77·2	41·2
E	Long	Meal	66·4	67·9	63·4	49·6	52·9	74·8	47·5
F	Ground	Flaked	53·5	56·6	63·4	38·1	15·4	64·6	13·3

* Diets B and D contained 40 % hay and 60 % concentrates whereas diets E and F contained 60 % hay and 40 % concentrates.

The same trends were discernible with the diets containing 40 % hay and 60 % concentrates containing maize meal. Grinding the hay, without changing the concentrates, caused a fall in the digestibility of dietary dry matter from 71·3 to 68·2 % and of the organic matter from 73·8 to 70·2 %. Again the effect was due mainly to a depression in the digestibility of crude fibre from 54·2 to 32·3 %.

The results of the digestibility trials were sufficient to show that the marked depression in the digestibility of the crude fibre due to grinding, observed when the hay used in the present experiments was given alone (Campling *et al.* 1963), also occurred when this hay was given with concentrates. Insufficient values were obtained to permit a precise estimate of the effect of grinding on the amount of digestible nutrients consumed daily by the cows and heifers in expts. 1 and 2, except that in expt. 2, part 1 it is probable that the digestibility of the organic matter was depressed over 10 percentage units. This suggests that in expt. 2, part 1 the intake of digestible organic matter fell by some 17 % when the hay was ground and the maize meal replaced by flaked maize. It is not possible, from the diets studied, to determine whether the depression due to grinding was greater with flaked maize than with maize meal.

DISCUSSION

The present experiments are chiefly of interest in demonstrating that diets that produced falls in milk fat percentage did not influence the yield of milk or the milk solids-not-fat content, neither was there any change in the rate of gain when the

diets were given to growing heifers. The marked depression in milk fat content frequently observed with ground roughage diets has usually been attributed to a change in ruminal production of propionic and acetic acids as reflected in the observed decrease in the ratio of acetic to propionic acid in rumen liquor. Such a hypothesis is certainly consistent with the marked effect of volatile fatty acid infusions on milk fat content (Rook & Balch, 1961). The infusion experiments demonstrated also, however, that acetic acid but not propionic or butyric acids increased milk yield and that propionic acid increased solids-not-fat content and produced a fall in milk fat content. Hence an increase in solids-not-fat content might be expected to occur in association with a decrease in fat content. This was not observed. In addition, Armstrong & Blaxter (1957) showed that the end products of digestion are used with a greater efficiency for fattening when they contain a high proportion of propionic and butyric acids than when they contain a high proportion of acetic acid. Diets that cause a depression in milk fat content in lactating cows could be expected therefore to give an improved food conversion in fattening animals. No such improvement was noted in the present experiment with growing heifers, but since grinding the hay and cooking the concentrates markedly decreased digestibility of the diet an improvement in utilization of digestible energy must have occurred.

One feature of the present experiments has been to emphasize the marked variability in the depression in fat content of the milk from cow to cow. This could be explained simply by variations from cow to cow in the relative proportions of volatile fatty acids in the rumen. Diet C, containing ground hay and flaked maize, used in the present series of experiments was given to each of 2 non-lactating fistulated cows at a rate of 15·5 lb dry matter daily. The observed molar proportions of individual acids in the total volatile fatty acids of rumen liquor were in one cow acetic 59, propionic 22, butyric 14, valeric 5 %, and in the other 50, 22, 22, 6 %. Corresponding values for diet A, containing long hay and flaked maize were 65, 18, 14, 4 % and 61, 19, 15, 5 %. D. A. Balch, however, reported an experiment (Balch, 1954) in which he found that the milk fat content of one cow receiving a diet containing ground hay was higher than that of a second cow receiving the same diet; it was noticed that although the proportions of volatile fatty acids were similar in the 2 cows the daily rise and fall in the concentration of the acids was less marked in the second than in the first cow. The possible implications of wide daily fluctuations in fatty acid production in relation to milk fat synthesis have been discussed by Annison & Lewis (1959).

The extensive literature on the effects of grinding the roughage of ruminant diets has been reviewed recently by Minson (1962). He concluded that with diets containing 50 % or more roughage and given at a fixed level of intake, the fine grinding of roughage and the cooking of concentrates in the diet, which sometimes decreased the digestibility of certain constituents, did not alter the rate of liveweight gain or carcass weight in growing animals: in milking cows fat percentage was usually depressed but milk yield was not affected. Increases in liveweight gain and in milk yield have, of course, frequently been observed when animals receiving ground diets consumed more than control animals receiving unground diets. The results of our experiments confirm Minson's conclusion. King & Hemken (1962) and Sutton, Jacobson, Erwin & Grainger (1962) have reported experiments in which milking cows received diets essentially similar to those used in expt. 1, the results of which conform

with our own. Shaw, Ensor, Tellechea & Lee (1960), however, reported that in an experiment with steers given a diet of hay, ground maize and linseed oil meal (1:1:0·04) finely grinding the hay and flaking the maize markedly increased rates of liveweight gain. In that experiment dry matter intake per 100 lb liveweight was maintained constant and in contrast to other experiments an increase in the digestibility of the protein of the diet was found that would account for a part of the increased efficiency of utilization of dietary energy. It should be recognized that grinding the roughage portion of mixed diets and changing the type of concentrates affects not only the ruminal volatile fatty acid production but also the rate of passage of food through the gut and the rate of breakdown of food in the gut. These various effects must clearly be fully understood before any serious attempt can be made to use the grinding of roughage or the cooking of concentrates as a method of increasing the efficiency of utilization of mixed diets for growth or milk production.

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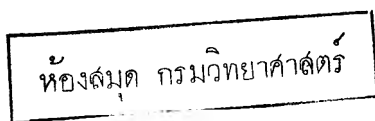
The effect of ultra-high-temperature heat treatment on the content of thiamine, vitamin B_6 and vitamin B_{12} of milk

By MARGARET E. GREGORY and H. BURTON

The National Institute for Research in Dairying, Shinfield, Reading

SUMMARY. Results are given for the effect of different ultra-high-temperature milk sterilizing processes on the thiamine, vitamin B_6 and vitamin B_{12} contents of milk. For all the processes examined, the loss of thiamine is negligible, and the losses of vitamins B_6 and B_{12} lie between zero and 35 %. Indirect-heating processes appear to cause more loss of vitamin B_{12} than of vitamin B_6, but the reverse is true of direct-heating processes.

Several different types of plant are now available for the ultra-high-temperature (UHT) sterilization of milk. They are being increasingly used to produce sterile milk which, when filled into containers by an aseptic process, will keep for several weeks without refrigeration. This development has led to interest on the part of users, manufacturers and health authorities in the probable effect of the different UHT treatment processes on the nutritive value of milk (e.g. Report, 1963).

The effects of pasteurization processes and of in-bottle sterilization processes on the nutritive value of milk are already known (Kon, 1960). A few results have also been published on the vitamin content of UHT sterilized milk (Chapman *et al.* 1957; Nagasawa, Tanahashi, Kuzuya & Shigeta, 1960; Lhussier, Hugot & Biette, 1962). However, a wider range of UHT processing methods is now available, in which the treatment temperature may be between 130 and 150 °C according to the method, and the effective time between 2 and 15 sec. Furthermore, two fundamentally different principles of heating are involved: indirect heating, in which heat is transferred to the milk through a stainless steel heating surface which takes the form either of a plate or a tube; and direct heating, in which steam is injected into the milk, or milk is sprayed into steam, so that the milk receives heat from the steam which is condensed. In the direct heating processes the milk is subsequently cooled by evaporation under partial vacuum, which removes the added water.

In view of the number of processes now available, it seemed desirable to find the effect on the vitamin content of milk for as many types as possible. The levels of those vitamins which are the most heat-labile in milk provide the best criteria for comparing different processes, and so the determinations were confined to vitamin B_6, vitamin B_{12} and thiamine.

Types of plant

Details of the plants from which samples were obtained, and their operating conditions as far as known, are given in Table 1.

Commercially operated sterilizers of both the plate and tubular indirectly heated types were tested. Only one tubular type plant was available, but milk from this plant was tested on two separate occasions (Table 1: 1a, b). Four plate-type plants, of the same make but of different detailed designs, were tested (Table 1: 2–5). The treatment temperatures were those in use at the dairies at the time the samples were taken. The treatment times were determined by the plant construction, but as with all plants using indirect heating, a considerable proportion of the heat treatment is during the heating period so that it is not possible to give an accurate effective holding time.

Table 1. *Details of UHT sterilizers studied*

Plant and sample no.	Type of plant	Throughout (gal/h)	Maximum temp. (°C)	Mean holding time (sec)	Date of sampling
			Treatment conditions		
(a) Indirectly-heated plants					
1a	Tubular	1500	135	15	July 1963
1b					Jan. 1964
2	Plate	500	127	10*	Mar. 1964
3	Plate	3600	132	10*	May 1964
4	Plate	1500	135	10*	May 1964
5	Plate	470	138	$3\frac{1}{2}$	Apr. 1964
6a	Lab. plate	12	131	3	Jan. 1964
6b			136		Jan. 1964
6c			140		Jan. 1964
(b) Directly-heated plants					
7a	Steam into milk	440	150	$2\frac{1}{2}$	Jan. 1963
7b					Nov. 1963
8a	Steam into milk	440	140	4	Nov. 1963
8b					Jan. 1964
9	Milk into steam	395	145	4*	May 1963

* Estimated.

For comparison with milk from these full-scale commercial plants, samples were also taken from a laboratory-scale plate heat exchanger, operated at 3 different temperatures (Table 1: 6a, b, c).

Samples were obtained from 3 plants using direct heating; each of these plants was made by a different manufacturer. In two of these, the milk was heated by the injection of steam under pressure. Both of these plants were sampled twice (Table 1: 7a, b, 8a, b). In the third plant, milk was sprayed into a steam chamber for heating. Because of the presence of a milk pool at the bottom of the heating chamber, it is difficult to state an exact holding time for this plant and an estimated figure only is given (Table 1: 9).

Sampling

The plants using indirect heating were all in the Midlands and south of England, and we were able to take samples ourselves. The untreated sample was taken from the raw milk balance tank, and the treated sample was taken at the plant outlet. Balance and float tanks were operated at a low level where possible, to minimize the hold-up of milk. The treated sample was taken at a calculated time after the untreated sample, determined by the milk flow rate and the volume capacity of the plant, so that the 2 samples represented the same bulk of milk as closely as possible.

Plants 1, 2, 3 and 4 were operating as pre-sterilizers before an in-bottle sterilizing process. Samples of treated milk could only be taken from these plants at a temperature of about 70 °C, but they were immediately cooled in running water. In plant 5, milk could be sampled at about 5 °C, and in the laboratory plant at about 25 °C.

Plants using direct heating methods are not permitted in Britain under existing legislation, so that samples from these had to be provided from abroad. In making the necessary arrangements, it was emphasized that the raw milk sample should correspond as closely as possible to the treated sample. The milks were transported by air, with the treated samples in aseptically filled cartons and the control samples either freeze-dried or packed in dry ice. The milk from all these plants was sampled cold.

Microbiological assay methods

For the determination of thiamine, 1 ml of milk or of reconstituted milk was mixed with 25 ml $\text{N}/30$ H_2SO_4 and held at 100 °C for 30 min. After cooling, the volume was made to 50 ml with water, the pH adjusted to 4·6 and the extract filtered. The filtrate was diluted to contain about 0·01 μg thiamine/ml, and the thiamine activity measured with *Lactobacillus fermenti* as described by Banhidi (1958).

Vitamin B_6 was measured with *Saccharomyces carlsbergensis* after autoclaving the milk samples with 0·055 N-HCl as described by Gregory (1959). For the determination of vitamin B_{12} the milk samples were heated in sodium acetate buffer (at pH 4·6) containing a trace of sodium cyanide and assayed with *Lactobacillus leichmannii* as described by Gregory (1954).

Each complete assay was repeated; each result given is the mean of the 2 assays.

The destruction of the vitamins expressed as a percentage of the original amount could be obtained directly when the control and treated samples were both liquid milks from the same bulk supply. Where the treated sample was liquid but the control sample freeze-dried, the total solids content of the liquid milk was determined and this value used to calculate the vitamin content/g of milk solids, from which the percentage loss of vitamin could be determined.

RESULTS AND DISCUSSION

The results for the thiamine, vitamin B_6 and vitamin B_{12} contents of the milks obtained from the different plants are summarized in Table 2. When repeat samples were obtained at different times from the same plants. i.e. 1*a, b*, 7*a, b*, 8*a, b*, the percentage losses for a vitamin were similar in the repeated tests.

Thiamine was almost completely stable in the different UHT treatments examined. Only one plant using indirect heating gave a loss, of only 10%. None of the plants using direct heating showed any loss of thiamine. This finding contrasts with the results of Lhussier *et al.* (1962), who found 20% loss of thiamine for directly-heated milk treated with a plant of the same type as no. 7.

The losses of vitamin B_6 were low with the indirect-heating processes, varying from zero to 12%. Although on one occasion one of the direct-heating plants gave no loss, a further sample from the same plant showed 14% loss and in other direct-heating plants there were losses of up to 35%.

Table 2. *Effect of ultra-high-temperature heat treatments on the thiamine, vitamin B_6 and vitamin B_{12} contents of milk*

Vitamin content of control milk, and percentage loss on treatment

Plant and sample no.	Thiamine		Vitamin B_6		Vitamin B_{12}	
	$\mu g/ml$	%	$\mu g/ml$	%	$m\mu g/ml$	%
(a) Indirectly-heated plants						
1 *a*	0·43	None	0·38	None	3·57	25
1 *b*	0·48	None	0·41	None	4·80	35
2	0·43	None	0·37	12	4·00	30
3	0·41	None	0·40	None	2·27	None
4	0·44	10	0·45	7	2·45	26
5	0·40	None	0·36	None	3·30	12
6 *a*	0·51	None	0·50	6	5·30	11
6 *b*	0·51	None	0·50	8	5·30	21
6 *c*	0·51	None	0·50	4	5·30	23
(b) Directly-heated plants						
7 *a*	—	—	3·88*	30	29·0*	28
7 *b*	4·27*	None	4·49*	25	37·7*	21
8 *a*	0·44	None	0·37	None	4·43	None
8 *b*	0·45	None	0·44	14	4·4	9
9	0·48	None	0·43	35	4·06	None

* Samples received freeze-dried. Contents given/g dried solids.

Losses of vitamin B_{12} for milk treated by the indirect processes varied from zero to 35%, with most of the commercial plants giving 20–30% loss. The direct-heating plants showed generally lower losses of vitamin B_{12}. A plant operating at the relatively high temperature of 150 °C gave 20–30% loss, but the other 2 plants, which were operating at 145 and 140 °C, respectively, gave less than 10%.

It appears that vitamin B_6 is more stable than vitamin B_{12} when milk is treated by the indirect-heating process, but that the reverse is true for direct-heating processes. In general, direct-heating plants operate at higher temperatures with shorter holding times than those using indirect heating, but this difference is not a satisfactory explanation for the results found in the present group of plants. One of the commercial indirect-heating processes (no. 5) and the laboratory plant (no. 6c) both operated under rather exceptional conditions of time and temperature which were very similar to those for the direct-heating plants, but they still showed more destruction of vitamin B_{12} than of vitamin B_6, whereas the direct-heating plants

showed the reverse. These results suggest that there is some fundamental effect of the method of direct heating by mixing with steam which causes the higher loss of vitamin B_6.

When the severity of heat treatment was increased by raising the operating temperature of the laboratory plate heater ($6a$, b, c), no significant losses of vitamin B_6 were observed, whereas the loss of vitamin B_{12} increased from 11 % at 131 °C to 23 % at 140 °C.

In general, no similar relation between vitamin destruction and severity of heat treatment could be recognized with the commercial plants. Even between apparently similar plants there were inconsistencies. For example, plant 2 was operating at a very low temperature at the time of sampling, but the losses of vitamins were higher than those in plant 3, operating at a higher temperature. The results for plant 4, operating at a still higher temperature, were similar to those for plant 2. These variations may represent incidental differences between similar plants or between the milks on which they operated. For example, the degree of aeration of the milk may be of considerable importance. Ford (1957) has shown that de-aeration can protect vitamin B_{12} in milk from destruction by heat, and an early investigation of the milk from a plant of type 7, at a time when a de-aerator formed a part of the apparatus, showed no loss of vitamin B_{12} (Report, 1957). This compares with 21–28 % loss in the present experiments, with a similar plant but no de-aerator.

All the UHT processes had a negligible effect on thiamine, but the losses of vitamin B_6 and B_{12} were higher than would occur during pasteurization. However, the losses were very much less than would be caused by an in-bottle sterilizing process.

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A note on the effect of a commercial drying process on the long chain fatty acids of milk

By J. H. MOORE and D. L. WILLIAMS

National Institute for Research in Dairying, Shinfield, Reading

The role of the essential fatty acids, linoleic and arachidonic acids, in human nutrition has been widely studied, particularly with respect to their possible action in preventing arterial disease in adults, e.g. Kinsell (1963). In addition, Hansen, Haggard, Boelsche, Adam & Wiese (1958) have emphasized the importance of linoleic acid in the nutrition of infants. Although milk fat contains only relatively small amounts of linoleic and arachidonic acids, the part played by milk and milk products in contributing essential fatty acids to the human diet has received considerable attention (Combes, Pratt & Wiese, 1962; Kon, 1962; Hansen *et al.* 1963; Garton, 1964). However, despite the initial dependence of many infants on dried milk as an exogenous source of essential fatty acids the effects of commercial drying processes on the constituents of milk fat have not been much investigated. A comparative study of the fatty acid compositions of raw and dried milk was therefore undertaken.

The dried milk was produced at the factory of Cow and Gate Ltd, Wincanton, by concentrating raw milk in a double effect plate evaporator to a total solids content of 27–29% and drying the milk concentrate on twin drum rollers. On each of 6 consecutive days, one sample of dried milk and one sample of the raw milk from which the dried milk had been manufactured were despatched in refrigerated containers to the laboratory at Shinfield. The total lipids were extracted from the samples of raw milk by an adaptation of the technique of Folch, Lees & Stanley (1957) devised by Nelson & Freeman (1959). The same procedure was used for the extraction of the lipids from the samples of dried milk after the latter had been 'reconstituted' by the addition of water (8 ml/g milk powder). The lipid extracts were washed with 0·88% (w/v) KCl (Folch *et al.* 1957) and were taken to dryness under reduced pressure by means of a rotary film evaporator connected to a supply of nitrogen. With minimum delay the lipids were dissolved in chloroform and the resulting solutions were filtered. Methanol was then added to give a final solvent composition of 2:1 (v/v) chloroform-methanol. Methyl esters of the milk fatty acids were prepared by the method of Stoffel, Chu & Ahrens (1959) and were analysed by gas-liquid chromatography on both polar (polyethylene glycol adipate) and non-polar (Apiezon L grease) columns as described by Moore & Williams (1963, 1964). The analyses were confined to fatty acids with 12 or more carbon atoms. The mean fatty acid compositions of the samples of raw and dried milk are given in Table 1 in which the shorthand designation of Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) is used to denote the individual acids. These results show that there was little difference between the fatty acid composition of the raw milk and that of the dried milk. In particular, it should be noted that the process of roller-drying did not appear to result in any loss of linoleic (18:2) and arachidonic

(20:4) acids. Nevertheless, the possibility that some geometrical isomerization of the unconjugated diene or tetraene structure occurred during this process cannot be entirely eliminated.

Table 1. *Fatty acid composition (molar percentage of the total fatty acids present with 12 or more carbon atoms) of raw liquid milk and of dried milk powder*

(Mean values of 6 samples with their standard errors.)

Acid	Raw liquid milk	Dried milk powder
12:0	1·2 ± 0·12	1·6 ± 0·13
14:0	9·6 ± 0·16	10·1 ± 0·34
14:1	0·7 ± 0·02	0·6 ± 0·04
15:0 br	0·5 ± 0·02	0·4 ± 0·03
15:1	0·3 ± 0·04	0·3 ± 0·03
15:0	1·1 ± 0·02	1·0 ± 0·03
16:0	34·4 ± 0·03	33·8 ± 0·24
16:1	1·5 ± 0·08	1·5 ± 0·08
17:0 br	0·6 ± 0·02	0·6 ± 0·04
17:1	0·5 ± 0·02	0·5 ± 0·02
17:0	0·7 ± 0·02	0·7 ± 0·02
18:0	13·8 ± 0·14	14·2 ± 0·29
18:1	30·8 ± 0·20	30·6 ± 0·15
18:2 ct, tt*	0·5 ± 0·07	0·4 ± 0·04
18:2	2·3 ± 0·06	2·3 ± 0·12
18:3	0·6 ± 0·06	0·6 ± 0·03
20:0	0·6 ± 0·08	0·6 ± 0·07
20:3	0·15 ± 0·03	0·10 ± 0·00
20:4	0·14 ± 0·02	0·17 ± 0·02

* Tentatively identified from relative retention times on Apiezon L columns as cis-trans and trans-trans isomers of conjugated octadecadienoic acid.

It is of interest to compare the results of the present investigation with those of Pol & Groot (1960), who found that the levels of linoleic, linolenic (18:3) and arachidonic acids (determined by alkali-isomerization analysis) were each 30–40 % lower in spray-dried milk than in lyophilized milk.

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Identification of a C_{20} multibranched fatty acid from butterfat as 3,7,11,15-tetramethylhexadecanoic acid

By R. P. HANSEN and F. B. SHORLAND

Fats Research Division, D.S.I.R., Wellington, New Zealand

and JAMES D. MORRISON

Division of Chemical Physics, C.S.I.R.O., Melbourne, Australia

SUMMARY. A C_{20} multibranched fatty acid earlier isolated from butterfat in trace amounts has been conclusively identified by means of mass and infra-red spectrometry and gas-liquid chromatography as 3,7,11,15-tetramethylhexadecanoic acid.

A C_{20} multibranched chain fatty acid was isolated in trace amounts from butter-fat by Hansen & Shorland (1951, 1953), and from hydrogenated ox perinephric fat by Hansen, Shorland & Cooke (1958), but its chemical structure was not determined. Later Bjurstam, Hallgren, Ryhage & Ställberg-Stenhagen (referred to by Stenhagen, 1961) and Sonneveld, Haverkamp Begemann, van Beers, Keuning & Schogt (1962) independently isolated from butterfat a C_{20} fatty acid with similar properties, which they conclusively identified as 3,7,11,15-tetramethylhexadecanoic acid. This acid was also detected by gas-liquid chromatography as a major constituent of ox plasma by Duncan & Garton (1963) and identified by Lough (1963, 1964). Recently Klenk & Kahlke (1963), and Kahlke (1963) followed by Hansen (unpublished work) have found this acid to occur in high proportions in the tissue and blood lipids of patients afflicted with the rare disease described as Refsum's syndrome.

The purpose of the present paper is to report that the C_{20} multibranched acid originally isolated in this laboratory from butterfat (Hansen & Shorland, 1951, 1953) has now been examined by mass and infra-red spectrometry and by gas-liquid chromatography and has been identified as 3,7,11,15-tetramethylhexadecanoic acid, thus confirming its identity with the fatty acid identified by Bjurstam *et al.* (1961) and by Sonneveld *et al.* (1962).

EXPERIMENTAL

The fatty acid identified in this work was fraction E 2 whose chemical and physical properties were earlier reported (Hansen & Shorland, 1953).

Mass spectrometric analysis. The mass spectrometer used was a 60° sector, 30 cm radius, single-focussing instrument. The sample was introduced by means of an all-glass greaseless inlet system, at 200 °C. The mass spectrum was recorded using magnetic scanning of the mass scale.

Infra-red analysis. The infra-red analysis was made with a solution of the fatty acid dissolved in carbon disulphide. A model 21 Perkin–Elmer spectrometer was used. Observed values were adjusted to the calibrated instrument.

Gas-liquid chromatographic analyses. Relative retention volumes (V_R) were determined on the methyl ester and are relative to methyl stearate. The gas-liquid chromatograph used was fitted with a ^{90}Sr detector as described by Lovelock, James & Piper (1959). The glass columns employed were 2·4 m in length and 6·5 mm in internal diam., and were packed with celite impregnated with (a) 20 % (w/w) polydiethylene glycol adipate and (b) 5 % (w/w) Apiezon L. Argon was used as carrier gas and the operating temperature was 207 °C.

Molecular rotations were calculated from optical rotatory dispersion curves determined on the Bellingham and Stanley/Bendix–Ericsson automatic recording spectropolarimeter, Polarmatic 62. The original instrument had been modified to plot rotation directly against wave number. The conditions used were: concentration 12·6 mg/ml; solvent, methanol; cell, 1 cm; expansion, 8 C; speed, 60.

RESULTS

Mass spectrum

In the mass spectrum of the methyl ester of this acid (see Fig. 1), the parent ion occurred at $m/e = 326$, and this fact, together with the relative abundance of the isotope peaks, indicated a molecular formula of $C_{21}H_{42}O_2$. A significant (M-31) peak
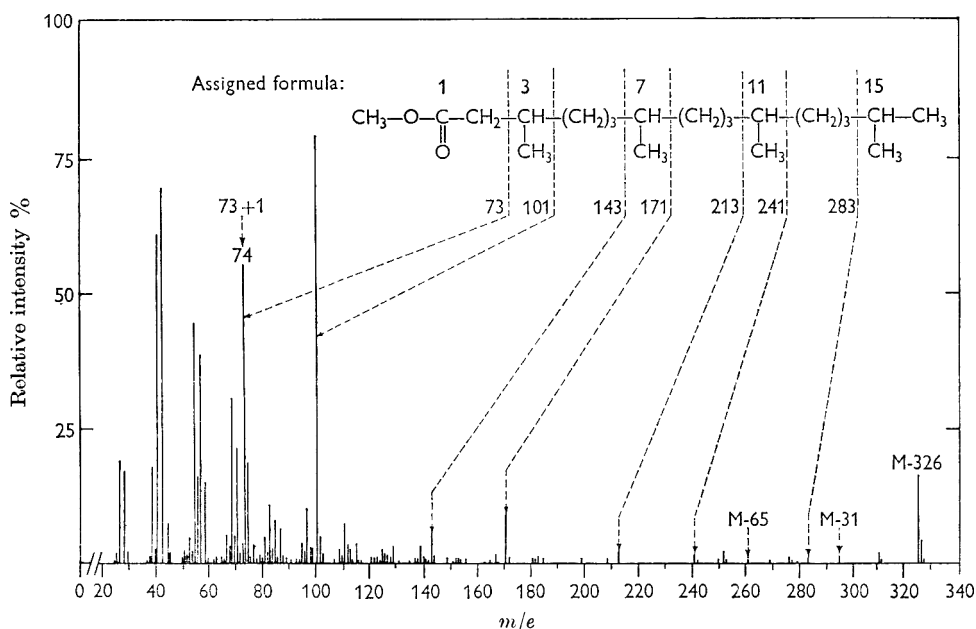
Fig. 1. Mass spectrum of methyl ester of C_{20} multibranched fatty acid (E_2).

confirmed that it was a methyl ester. The remainder of the structure could be readily deduced following the rules derived by Ryhage & Stenhagen (1960 *a*, *b*). A prominent re-arrangement peak at mass 74 indicated the grouping

$$CH_3\text{—}O\text{—}C\text{—}CH_2\text{—}$$
$$\overset{\|}{O}$$
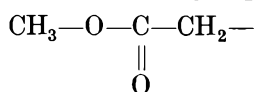
The base peak occurred at $m/e = 101$, while the peaks at 143, 171, 213 and 241 were very significantly larger than those adjacent at 87, 157 and 227. This suggested that there were methyl groups attached at positions 3, 7 and 11. Re-arrangement ions of ketene type

$$O\!=\!C\!=\!CH\!-\!\underset{\underset{CH_3}{|}}{CH}\!-\!\left[\underset{\underset{CH_3}{|}}{(CH_2)_m\!-\!CH}\right]_n^+$$
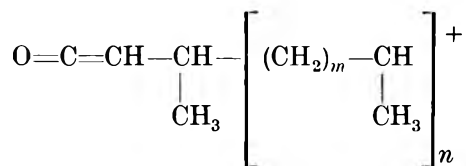
were to be expected, and were found at masses 139, 209 and 279 supporting the postulated methyl substitutions at C$_7$ and C$_{11}$ and suggesting a fourth substitution at C$_{15}$. A small peak at M-65 confirmed the presence of a terminal isopropyl group.
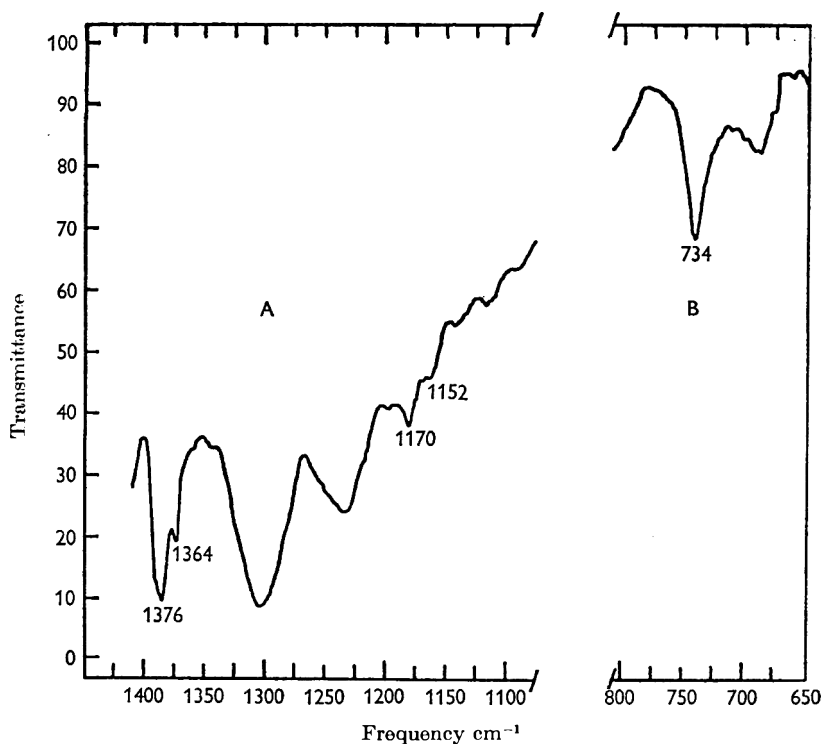
Fig. 2. Infra-red spectrum of C$_{20}$ multibranched fatty acid (E$_2$). A, 1100–1400 cm^{-1}; B, 650–800 cm^{-1} (calibrated values).

The mass spectrometric examination suggested therefore that the structure was

$$CH_3\!-\!\underset{\underset{CH_3}{|}}{CH}\!-\!(CH_2)_3\!-\!\underset{\underset{CH_3}{|}}{CH}\!-\!(CH_2)_3\!-\!\underset{\underset{CH_3}{|}}{CH}\!-\!(CH_2)_3\!-\!\underset{\underset{CH_3}{|}}{CH}\!-\!CH_2\!-\!\underset{\underset{O}{||}}{C}\!-\!O\!-\!CH_3$$
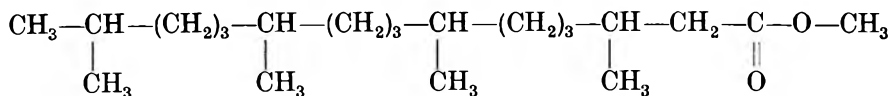
and therefore identical with the methyl ester of the acid isolated from butterfat and identified by Bjurstam *et al.* (1961) and by Sonneveld *et al.* (1962).

Infra-red spectrum

The infra-red spectrum of the methyl ester of this acid (Fig. 2) showed the following absorption characteristics of a terminal isopropyl grouping: (*a*) a strong band at 1170 cm^{-1} with a shoulder at 1152 cm^{-1} (Simpson & Sutherland, 1949; Pliva & Sörensen, 1950; Bendoraitis, Brown & Hepner, 1962), and (*b*) a doublet at 1364 and 1376 cm^{-1} (Thompson & Torkington, 1945). Also present was a strong band at 734 cm^{-1} (unaccompanied by a prominent shoulder at 727 cm^{-1}) indicating a regular triple CH_2 sequence (Pliva & Sörensen, 1950; McMurray & Thornton, 1952; Bendoraitis *et al.* 1962). If the regular $(CH_2)_3$ sequence had been interrupted by one irregular $(CH_2)_4$ grouping there would have been a prominent shoulder at 727 cm^{-1} (Pliva & Sörensen, 1950), but such was not the case. Confirmation of the terminal grouping as methyl and not ethyl was inferred from the absence of a moderately strong band at 770 cm^{-1} which characterises a terminal ethyl structure (Thompson, 1948; Pliva & Sörensen, 1950).

Gas-liquid chromatographic analyses

When analysed by gas-liquid chromatography using the polydiethylene glycol adipate column at 207 °C, the methyl ester of this multibranched C_{20} acid had a relative retention volume (V_R) of 0·72, corresponding to a 'carbon number' (Woodford & van Gent, 1960) of 16·9. With the Apiezon L column at 207° the V_R was 0·81 which represents a 'carbon number' of 17·6. Gas-liquid chromatography indicated this acid to be 99·5 % pure, the trace of impurity being palmitic acid.

Molecular rotation

The following molecular rotations were recorded for the methyl ester: 233 mμ, +70; 250 mμ, +45; 300 mμ, +8; 400 mμ, +4; 500 mμ, +4.

<div align="center">DISCUSSION</div>

The C_{20} multibranched fatty acid isolated from butterfat (Hansen & Shorland, 1951, 1953) is conclusively identified by its mass spectrum as 3,7,11,15-tetramethylhexadecanoic acid. Apart from this evidence, the infra-red spectrometric analysis, which implies an isopropyl terminal grouping and a regular triple methylene sequence, when considered in conjunction with the saponification equivalent of 312 (Hansen & Shorland, 1953) also indicates that the chemical constitution is 3,7,11,15-tetramethylhexadecanoic acid.

The recent isolation from butterfat of a C_{19} multibranched fatty acid 2,6,10,14-tetramethylpentadecanoic acid (Hansen, 1964; Hansen & Morrison, 1964) suggests that a series of these acids may exist in trace amounts, and may be widely represented in nature.

The structural similarities of 3,7,11,15-tetramethylhexadecanoic acid, 2,6,10,14-tetramethylpentadecanoic acid, phytol, and pristane indicate a close biochemical relationship amongst these substances. Phytol, which comprises about 30 % of the chlorophyll molecule, suggests itself as the precursor of all 4 substances. Pristane is of widespread though not abundant occurrence, and its sources point to its origin in plants. Accompanied by smaller amounts of the unsaturated hydrocarbon zymene, it is a constituent of the liver oils of sharks (Tsujimoto, 1917; Toyama, 1923; Sörensen & Mehlum, 1948) and of whales (Tsuchiya & Kaneko, 1951) from which

latter it is extracted commercially. It is also present in planktonic crustaceans (*Calanus* sp.) (Blumer, Mullin & Thomas, 1963), in herring oil (Hallgren & Larsson, 1963) and in ambergris (Lederer & Pliva, 1951). In addition, pristane has been found in crude petroleum (Bendoraitis *et al.* 1962), coal tar (Kochloefl, Schneider, Řeřicha, Horák & Bažant, 1963), and wool grease (Mold, Stevens, Means & Ruth, 1963).

To explain the origin of pristane in shark liver oil, Sörensen & Sörensen (1949) consider 'phytanic' acid (3,7,11,15-tetramethylhexadecanoic acid) to be intermediary between phytol and pristane. These authors postulate that the enzymatic processes of the bacterial flora of the zooplankton, or of other animals higher in the food chain, dehydrogenate the primary alcoholic group of phytol to a carboxyl group and at the same time hydrogenate the allylic group to yield 'phytanic' acid, followed by decarboxylation to form the isoprene hydrocarbon pristane. This hypothesis is supported by the discovery of appreciable amounts of pristane in zooplankton crustaceae (*Calanus* sp.) (Blumer *et al.* 1963) and is consistent with the derivation of other lipid constituents, such as vitamin A, in the larger marine animals by direct ingestion of these substances already present in the zooplankton (Fisher, Kon & Thompson, 1952). Similarly, it is conceivable that in the cow, enzymatic reactions of certain rumen micro-organisms are responsible for the conversion of phytol from ingested pasture to the C$_{20}$ tetramethyl fatty acid while others produce the C$_{19}$ homologue. It might be expected that such synthesis would be preferential and within the microbial tissues, and the presence in butterfat of these 2 fatty acids could result from assimilation of the lipids of these micro-organisms. The detailed work of Keeney, Katz & Allison (1962), which involved studies on the amounts and the range of branched-chain acids in rumen bacterial and protozoan lipids, suggests that rumen micro-organisms are the main source of the *iso* and *anteiso* acids found in the milk and depot fats of the cow (Shorland & Hansen, 1957). This view was expressed by Akashi & Saito (1960) and supported by the work of Saito (1960*a, b*), Macfarlane (1961*a, b*, 1962), and Lennarz (1961). The C$_{20}$ multibranched fatty acid 3,7,11,15-tetramethyl-hexadecanoic acid has not been reported in the lipids of rumen micro-organisms, but its detection by gas-liquid chromatography when present in small amounts could readily be obscured by the methyl esters of other constituents, including methyl heptadecanoate, with similar retention volumes.

The evidence discussed by Abrahamsson, Ställberg-Stenhagen & Stenhagen (1963) relating to the configuration of 3,7,11,15-tetramethylhexadecanoic acid isolated from butterfat by Hansen & Shorland (1953) together with its specific rotation ($[\alpha]^{18.5\circ}$, +1·1 in chloroform) in comparison with that of naturally occurring phytol ($[\alpha]_D^{18\circ}$, +0·20-0·21°; Karrer, Geiger, Rentschler, Zbinden & Kugler, 1943) is not inconsistent with its biosynthesis from this alcohol.

Further information on bacterial biosynthesis of branched-chain fatty acids is provided by Prof. R. E. Kallio (personal communication), whose evidence indicates that a great number of bacteria utilize pristane as a sole source of carbon and energy, and that in this process pristane appears to be first converted to the C$_{19}$ multibranched acid (2,6,10,14-tetramethylpentadecanoic acid) and subsequently to α-methyl glutaric acid.

Comparison of the trace amounts of 3,7,11,15-tetramethylhexadecanoic acid found in butterfat with the relatively large amounts present both in the lipids of humans with Refsum's syndrome (Klenk & Kahlke, 1963; Hansen, 1964) and in the blood

lipids of ox (Duncan & Garton, 1963; Lough, 1963, 1964) prompts consideration of an alternative biosynthetic mechanism referred to by Sonneveld *et al.* (1962) and based on the enzymatic conversion of mevalonic acid into 'prenoic' acids. Moreover, biosynthetic routes are notably varied so that the possibility of different syntheses of the isoprenoid acids should not be overlooked.

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A thermoduric strain of *Streptomyces albus,* isolated from Cheddar cheese

By T. F. FRYER and M. ELISABETH SHARPE

National Institute for Research in Dairying, Shinfield, Reading

SUMMARY. A strain of *Streptomyces albus*, presumed to have originated in raw milk, was found in large numbers in a pair of experimental cheeses. The numbers of streptomyces in both cheeses remained constant over the 4-month period of sampling and a comparison of the numbers obtained from cheese dissolved in citrate with and without mechanical aid revealed no differences, suggesting that the streptomyces was present in the conidial state. The heat resistance of mycelium and of conidia was determined at 62·7 °C (145 °F) and 71·7 °C (161 °F). The mycelial cells showed no resistance to either temperature, there being 100% kill after 15 min at 62·7 °C and 17 sec at 71·7 °C. The conidia, however, were very much less affected after 2 h at 62·7 °C, and 3% remained viable even after 30 min at 71·7 °C. Although the strain was found to be proteolytic, lipolytic and saccharolytic, it appeared to be inactive in the cheese, being present in the conidial state.

Streptomyces, which are of soil origin, have been found in the air of the cowshed, on cows' udders, in milking machines and in raw milk (Hlaváčková, 1951) and might therefore be expected to occur in cheese. Bernstein & Morton (1934) described a thermophilic actinomycete which they isolated frequently from pasteurized cheeses, but the occurrence of a streptomyces in relatively large numbers (10^4/g) in Cheddar cheese, and survival in the cheese for several months has not been reported previously. During a study of the microflora of cheese made from heat-treated milk at the Experimental Dairy at this Institute a strain identified as *Streptomyces albus* was found in a pair of cheeses. As aerial and utensil contamination was excluded from one of the cheeses, the streptomyces must have originated from the raw milk and withstood the heat treatment of 71·7 °C. for 17 sec. This suggests an unusually high heat resistance for a streptomyces (Waksman, Umbreit & Cordon, 1939). As the presence of such an organism might have some effect on the flavour of the cheese, its physiological characteristics, heat resistance, and inhibitory effect on other micro-organisms likely to be present in cheese were investigated. The incidence of the streptomyces in subsequent samples of milk from the same herd, and in a number of other cheeses made from it was also determined.

METHODS

Cheesemaking

The 2 cheeses, 'aseptic' and control, made as part of our investigations on cheese flavour, were each made from 40 gal of single-herd high quality milk, heat-treated at 71·7 °C for 17 sec; the 'aseptic' cheese being made in a specially adapted vat using the techniques of Mabbitt, Chapman & Sharpe (1959). Whereas all extraneous organisms other than starter streptococci and those able to survive heat treatment of the milk were excluded from the 'aseptic' cheese, contamination of the control cheese could occur after heat treatment.

Sampling of milk and cheese and the isolation of streptomyces

Milk

Milk samples from a single herd were examined on 31 occasions over a period of 9 months for the presence of streptomyces. On each occasion duplicate raw milk samples were taken from the farm tank, from the dairy mixer tank and from the pipeline as the heat-treated milk entered the control cheese vat.

Cheese

Cheese was sampled according to the method described by Naylor & Sharpe (1958). Serial dilutions, in quarter-strength Ringer's solutions, of milk and of cheese homogenized in 2 % citrate were plated in deep plates of nutrient agar which were incubated at 30 °C for 5 days, and then left at room temperature for 2–3 days. Colonies of streptomyces recognized by their appearance were enumerated and picked off into a suitable medium. In addition to the pair of cheeses found to contain streptomyces, 9 other cheeses made within a few days of this pair, from milk from the same source, were also examined at different ages. The incubation temperature for all tests and culturing was 30 °C unless otherwise stated.

Cultural medium

At first the organism was grown in nutrient broth, but a comparison of the growth in 4 media, viz. nutrient broth, Yeastrel nutrient broth (YNB), glucose nutrient broth and Yeastrel glucose nutrient broth showed that YNB gave the best growth. This medium was used for general cultural work.

Preservation of strains

Strains were stored at 4 °C on agar slopes of the ammonium lactate medium (AL) of Gyllenberg, Eklund, Antila & Vartiovaara (1960). Growth on this medium resulted in an abundant yield of conidia which facilitated subculturing.

Heat resistance of mycelium and conidia

Preparation of mycelium free from conidia

Tubes containing 5 ml of YNB were inoculated with growth from the AL slopes and incubated in a sloped position for 24 h, the increased aeration encouraging both growth of mycelium and germination of conidia. Three successive transfers were made

in this manner, using 0·1 ml inoculum. It was necessary at each subculture to ensure that none of the inoculum ran down the side of the tube, in order to prevent the growth and sporulation thereupon of individual colonies of streptomyces.

One tube was selected in which no aerial mycelium was evident and the mycelial aggregates were broken up using a sterile Griffiths' tube. The mycelial fragments were centrifuged, washed once in saline and resuspended in 5 ml of sterile skim-milk. This culture was incubated in the sloped position for 4 h, so that cells damaged by homogenization might have a chance to recover.

Preparation of conidial suspension

After 7-days' incubation, the growth of 5 AL agar slopes was harvested into 2 ml of skim-milk containing a wetting agent 2 % Tween 80. The suspension was homogenized in a Griffiths' tube.

Heat resistance

This was determined by a modification of the technique of Stern & Proctor (1954) described by Franklin, Williams & Clegg (1958). The temperatures used were 62·7 °C (145 °F) and 71·7 °C (161 °F) being those commonly used for the pasteurization of milk, with a range of times. A comparison of a number of media showed that recovery of the heat-shocked cells was most successful on nutrient agar containing 10 % skim-milk. The number of survivors was estimated by the Miles & Misra (1938) technique, the plates being incubated for 3 days in the case of the mycelium and 5 days for the conidia. A longer incubation period was required for the conidia as some of them showed a considerable lag before germinating.

Physiological tests

0·05 ml of an 18–24 h culture of the organism grown in 5 ml YNB, centrifuged and resuspended in 5 ml distilled water was used as inoculum. Two isolates from the 'aseptic' cheese and two from the control cheese were examined.

Optimum growth temperature

Inoculated nutrient agar slopes were incubated at 10, 22, 30 and 37 °C for 2 weeks.

Biochemical tests

Hydrolysis of gelatin, starch and casein, reduction of nitrate and fermentation of sugars were based mainly on the methods of Gordon & Smith (1955) and Gordon & Mihm (1957): Lipolysis was tested on a tributyrin agar medium (Franklin & Sharpe, 1963) and a butterfat agar (Jones & Richards, 1952). Growth in litmus milk over a period of 5 weeks was also examined.

Inhibitory effects

To observe the inhibitory effects of the streptomyces against bacteria commonly present in milk or cheese, a thick layer of nutrient agar was poured into a large Petri dish (15 cm ext. diam.) and a large segment of agar cut out leaving the small segment

(about 3 cm at the widest part) which was inoculated with streptomyces. After 2-days' incubation a thin layer of an agar appropriate to culture the test organism was poured into the empty portion and the test organism, grown in an appropriate broth medium, streaked across the plate at right angles to the streptomyces (Fig. 1). Incubation was continued for a further 2 days and any antagonistic effects noted. The streptomyces culture was grown on nutrient agar throughout the test so that any change in antibiotic production which might occur if grown on the media used for the test organisms was eliminated. Growing the streptomyces on isolated segments of agar prior to testing their antagonistic properties allowed any antibiotic to accumulate and precluded any change of the media upon which the test organisms were to be streaked.
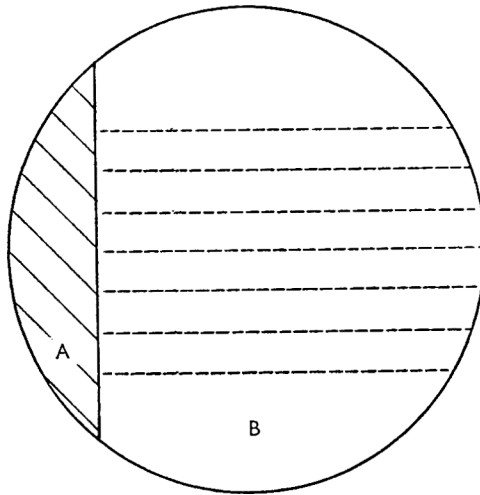
Fig. 1. Diagrammatic illustration of technique used to assess antibiotic production by *Str. albus* isolated from cheese. A, Original segment of nutrient agar inoculated with streptomyces culture and then incubated for 5 days; B, remaining segment of plate then filled with appropriate culture medium and test organisms streaked on the surface.

Lactobacilli, pediococci and leuconostocs were cultured in MRS broth (deMan, Rogosa & Sharpe, 1960) and MRS agar was used in the test plate; groups D and N streptococci were cultured in glucose Lemco broth and grown on yeast glucose agar; coagulase positive staphylococci, Gram-negative rods and micrococci were cultured in nutrient broth and nutrient agar was used in the test plate.

RESULTS

Enumeration of streptomyces in raw and heat-treated milk

Most of the duplicate samples of 31 raw milks and the corresponding heat-treated milks (71·7 °C for 17 sec) examined contained streptomyces in numbers of 1–50/ml. The numbers present in raw and heat-treated milks showed little difference.

Enumeration of streptomyces in cheese

After 2-weeks' ripening the numbers of streptomyces obtained in the 2 cheeses were identical, being 3.0×10^4/g. After 4 months, counts of 1.6×10^4/g and $1.7 \times 10/^4$g were obtained for the aseptic and control cheeses, respectively. No streptomyces were found in the 9 other cheeses sampled, and if present were therefore in numbers less than 5×10^2/g.

Heat resistance tests in the laboratory

Mycelium

There were no survivors after 15 min at 62.7 °C or after 17 sec at 71.7 °C. The unheated control had a count of 8.75×10^5/ml.

Conidia

There appeared to be little destruction of the conidia within the experimental error of sampling even after 2 h at 62.7 °C, whilst after 30 min at 71.7 °C only 3 % still remained viable (Table 1).

Table 1. *Survival of streptomyces conidia after heat treatment at different temperatures for different times*

Heat treatment		No. of streptomyces/ml	% kill
Unheated control		10.5×10^6	
62.7 °C (145 °F)	5 min	3×10^6	71.4
	15 min	9×10^6	14.3
	30 min	6×10^6	42.8
	60 min	6×10^6	42.8
	120 min	4.5×10^6	57.1
Unheated control		10.1×10^6	
71.7 °C (161 °F)	15 sec	10.0×10^6	1
	30 sec	5.5×10^6	45
	1 min	6.5×10^6	35
	2 min	5.4×10^6	30
	15 min	8.5×10^5	92
	30 min	3.5×10^5	97

Comparison of the counts of streptomyces in homogenized and unhomogenized cheese

Skinner (1951), working with soil, observed that when suspensions containing a preponderance of vegetative mycelium were shaken a large increase in numbers occurred, whereas when spores were chiefly present no increase occurred. When the numbers of streptomyces found in homogenized cheese were compared with those found in unhomogenized cheese (grated and suspended in warm 2 % potassium citrate) no differences were observed.

Physiological and biochemical tests

Colony appearance

The isolates all produced white growth on solid media.

Optimum growth temperature

Although growth occurred more rapidly at 37 °C than at 22 or 32 °C the appearance of the growth was very different, very little aerial mycelium being visible at 37 °C, whilst at 30 and 22 °C normal growth of aerial mycelium occurred.

Biochemical tests

Gelatin, starch and casein were all strongly hydrolysed. Visible zones of casein hydrolysis appeared in 3 days even when grown on 50% skim-milk nutrient agar. It was also found that as the proportion of skim-milk to nutrient agar was increased the formation of aerial mycelium was delayed. When grown on 20% skim-milk agar, aerial mycelium did not become apparent until after 15 days, and then occurred only at points where the colonies were crowded together. On ordinary nutrient agar all colonies showed sporulation after 4 days.

Nitrate was reduced slowly, a strong positive reaction being obtained only after 14 days; lipolysis was demonstrated both on tributyrin agar and on butterfat agar, the latter medium being only weakly hydrolysed. Litmus milk was coagulated rapidly and later digested. The 4 isolates proved identical in all reactions and were identified as *Str. albus*.

Table 2. *The antagonistic effect of Streptomyces albus isolated from cheese on strains of micro-organisms isolated from milk and cheese*

Organism	No. of strains examined	No. of cultures showing inhibition	Zone of inhibition by positive cultures, cm
Lactobacillus casei	3	3	1–2
L. plantarum	3	3	1–2
L. brevis	3	3	1–2
L. buchneri	2	2	1
Lactobacilli (unclassified streptobacteria)	3	2	1–2
Leuconostocs	3	2	1
Pediococci	3	0	0
Group D streptococci	8	0	0
Group N streptococci			
(a) *Str. lactis*	3	0	0
(b) *Str. cremoris*	3	2	1–2
Micrococci	12	6	1–2
Gram-negative rods	10	3	1–2
Coagulase positive staphylococci	6	0	0

Antibiotic formation

None of the test organisms was strongly inhibited (Table 2), the inhibition, when present, usually being between 1–2 cm. Tests by the Antibiotics and Fermentation Division of Boots Pure Drug Co. showed that the amounts of antibiotic produced were too small to warrant further investigation.

DISCUSSION

Although the mycelium of this strain of *Str. albus* was sensitive to heat, the conidia proved to be highly resistant. Lutman & Cunningham (1914) observed little difference between the low thermal death point of mycelium and conidia of a strain of *Streptomyces scabies*, and Waksman *et al.* (1939) confirmed this observation with over 100 mesophilic strains of streptomyces, most of which were killed by heating at 50 °C. Inverso & Weiser (1949) found that sporulating cultures suspended in skim-milk were also destroyed by heating for 10 min at 65 °C. Apart from Erikson's (1955 *a, b*) reports of a strain of *Str. albus* able to resist 90 °C for 1 min, and the ability of the thermophilic actinomycete *Micromonospora vulgaris* to survive 4 h at 100 °C there has previously been little evidence of heat-resistant actinomyces. The ability of the conidia of the strain described here to survive for 2 h at 62·7 °C or for 30 min at 71·7 °C indicates an unusually high heat resistance.

In the raw milk the organism must have been present in the conidial form in order to survive the heat treatment to which it was subjected (71·7 °C for 17 sec). In the 2 cheeses the consistent numbers observed over a period of 4 months and the lack of evidence of fragmentation on homogenization of the cheese suggest that here also the streptomyces was present as conidia and not mycelium. Proliferation of so strict an aerobe in the cheese would be unlikely so that despite its proteolytic, lipolytic and saccharolytic properties, it is doubtful whether its presence in cheese even in large numbers would affect the flavour. In fact the flavour of the 2 cheeses was very similar to other experimental cheeses in the same series.

As it was not growing in the cheese, the large numbers present must have been due to heavy contamination of the raw milk with the heat-resistant streptomyces spores. The absence of the organism from 9 other cheeses made within a few days of the contaminated pair, and the presence of only small numbers in milks examined over a period of 9 months suggest that this was an unusually high contamination of the milk supply.

Whilst the semi-anaerobic conditions found in cheese would inhibit growth of the streptomyces it is not known whether proliferation would occur on cut surfaces of cheese exposed to the air, or within cheeses where cracks have occurred and allowed oxygen to permeate.

The detection, on a different occasion, of a streptomyces in a commercial starter streptococcus culture suggests that it might also enter cheese in this way.

The inhibition observed against some common dairy organisms such as some of the starter streptococci, might under some conditions influence the milk or cheese microflora.

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The nature and cause of seaminess in Cheddar cheese

By J. CONOCHIE and B. J. SUTHERLAND

Division of Dairy Research, C.S.I.R.O., Melbourne, Australia

SUMMARY. Microscopical and chemical studies of seaminess in Cheddar cheese revealed that the white lines or seams characteristic of the defect are sections through layers of crystals lying between the milled curd particles. The crystals were identified from their X-ray diffraction pattern and by their refractive index as calcium ortho-phosphate dihydrate, $CaHPO_4.2H_2O$. On each side of the adjoining curd surfaces in affected cheese there is a zone about 20 μm thick of strongly contracted protein which is almost devoid of crystals.

Adding sodium chloride to cheddared curd increased the quantities of calcium, phosphorus and water released. The increases were proportional to the amount of salt applied within the range 0–2·5 g NaCl per 100 g curd.

The solubility of calcium orthophosphate was found to rise from about 0·0025 M in water to a maximum of 0·008 M in 2 M sodium chloride solution.

It is postulated that calcium and phosphate ions released from the curd into the seam crystallize in the form of $CaHPO_4.2H_2O$ as the solubility of the compound is lowered by diffusion of salt from the surfaces into the curd particles.

INTRODUCTION

Seaminess is a condition in Cheddar cheese in which the junctions of the milled curd particles are visible after pressing. In extreme cases it persists after the cheese has matured (Plate 1).

The phenomenon is regarded as a defect because of the uneven colour and because the junctions are usually weak, due to incomplete fusion. This often leads to crumbling when the cheese is sliced or cut into small blocks for packing. The defect is accentuated by exposure to dry conditions after the package is opened. Van Slyke & Price (1952) state that the condition is caused by 'greasy curd, impure salt, too rapid application of salt and rapid evaporation of moisture from the curd surfaces'. They suggest it can be prevented by rinsing the curd with warm water, by using clean salt added in several applications and by keeping the vat covered to reduce evaporation.

Czulak (1963) found that the defect could be eliminated by spraying the curd before salting with a mist of warm water. The amount of fat removed by this treatment was negligible, suggesting that a cause other than a film of fat is responsible. Czulak concluded 'it is more likely that seaminess is caused by some alteration in the surface layer of the curd particle, possibly as a result of its severe dehydration on contact with dry salt'.

This paper presents the results of a study of the nature and cause of seaminess and of the effect of salt on the fusion of the curd.

Microstructure of the seams

Sections 10–20 μm thick were cut with a microtome through seams in pieces of cheese frozen with liquid carbon dioxide. The sections were examined microscopically under ultra-violet light with a yellow filter after staining with acridine orange dye (1 in 1000 w/v in phosphate buffer pH 6·8) or under polarized light after mounting in water.

Composition of the body and seam

Cheese from seams and from the body of the curd was analysed for moisture, fat and protein by micro methods.

Moisture was measured by titration with Karl Fischer reagent using an electro-metric end-point indicator. Samples of about 30 mg were weighed on pieces of Teflon which were transferred with the cheese to the titration vessel.

Fat was estimated by the hydroxamate method of Nijs & Verheyden (1960). A sample of 80–90 mg of cheese was ground with 1·5–2·0 g of washed sand in an agate mortar and transferred to a 25-ml volumetric flask with ethyl ether. A 1-ml aliquot was used for each determination. Protein was determined by the micro-Kjeldahl method, employing a copper catalyst and distilling into boric acid.

Effect of salts

Various salts ($NaCl$, Na_2SO_4, $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, $BaCl_2$) were spread evenly over slices of curd which had been cheddared under vacuum to eliminate cavities. These slices were then pressed to form cheeses. The amounts of salt were increased from slice to slice and covered a range equivalent to that of $NaCl$ in commercial Cheddar cheese. After 13 days the effects on seaminess and fusion were judged subjectively on slices cut at right angles to the salted surfaces. Thin slices were cut from the face with a wire and the slices bent along the seam to determine the nature and location of fracture and hence the extent of fusion.

Effect of insoluble material on fusion

Tests were made on the effect on fusion of washed sand sieved to a grain size comparable with that of the sodium chloride used in previous tests.

Identification of crystals in the seams

The crystalline material occurring in the seams of commercial Cheddar cheese (Plate 3 (2)) was examined by X-ray diffraction. X-ray photographs were taken with a micro-beam X-ray camera similar to that designed by Chesley (1947). A microtome section of a seam was placed across the 100 μ aperture of the camera. An X-ray pattern was obtained from normal cheese for comparison. The refractive index of the crystals was determined microscopically by the use of reference oils.

Release of moisture, calcium and phosphorus from cheddared curd treated with sodium and calcium chlorides

The amounts of moisture, calcium and phosphorus released from cheddared curd by the application of NaCl and of $CaCl_2$ were measured as follows.

Slices $\frac{3}{8}$ in. thick and 2 in. diam. and weighing approximately 25 g were cut from cheddared curd. After removal of free moisture with a filter paper, the salts were spread evenly with a spatula over the circular curd surfaces. Filter papers were then placed over each surface of the slice which was held at 30 °C in a covered stainless steel moisture dish for 70 min. Controls of unsalted curd slices and of filter papers alone were included. After the interval of 70 min the filter papers were pressed firmly against the curd to absorb free liquid and the curd slices were removed. The amount of moisture absorbed by the papers was then determined by weighing, drying, equilibrating to atmospheric conditions and reweighing.

For the determination of calcium and phosphorus released, the filter papers and dishes were washed with 100 ml distilled water at 50° C. A check on further 50 ml washings showed that at least 98 % of the calcium was contained in the first 100 ml.

Calcium was determined by the indirect EDTA method of Sawyer & Hayes (1961) and phosphate by the method of Allen (1940).

The solubility of $CaHPO_4.2H_2O$ in salt solutions of various strengths

Pure $CaHPO_4.2H_2O$ was prepared according to the method of Lugg (1931) and its identity and purity checked by X-ray diffraction and by conversion of the hydrate to pyrophosphate.

The solubility in salt solutions of various strengths was then determined by shaking 3 g of $CaHPO_4.2H_2O$ with 50 ml of salt solutions at 20 °C for 24 and 48 h, the pH and content of Ca^{++}, PO_4''' and Cl' being determined on a filtrate through a Whatman No. 41 paper.

Calcium was determined by the direct method of Sawyer & Hayes (1961) and phosphate by the method given above. Chloride was determined by titration with silver nitrate and pH was measured with a glass electrode and calomel half-cell.

<center>RESULTS</center>

Composition of body and seam

Using the micromethods described by which thin scrapings were analysed, no differences greater than 0·5 % in moisture, 1·0 % in fat and 0·2 % in protein content were detected between seam and body material. These differences are insignificant as they are within the sensitivity of the analytical methods.

Microstructure of the seam

In a section of a seamy cheese stained with fluorescent acridine and viewed under ultra-violet light with a yellow filter, the fat appeared green and the protein orange. As shown in Plate 2 (1), a layer about 20 μm wide could be seen on each side of the seam. This layer appeared much denser and relatively devoid of fat. Beyond this layer numerous lakes of fat were enmeshed in the protein network. The dense layer

seen in Plate 2 (1) is only apparently well fused, in fact there runs through it a line of weakness as is evident in Plate 2 (2), which shows another part of the same seam.

Under polarized light normal Cheddar cheese showed an even distribution of crystals through the body of the cheese as in Plate 3 (1), while seamy cheese (Plate 3 (2)) showed a heavy agglomeration of crystals along the entire length of the seam.

Effect of various salts on seaminess and on fusion

The effects of various salts on the extent of seaminess and on fusion are shown in Table 1.

Table 1. *The effect of various salts on seaminess and fusion in Cheddar cheese*

Salt	Salt concentration g/cm^2 curd surface	Seaminess	Extent of fusion
NaCl	0·011–0·026	None to severe	Fair to weak
Na$_2$SO$_4$	0·0–0·032	Severe	Absent
MgCl$_2$	0·0–0·046	None	Weak
MgSO$_4$.7H$_2$O	0·022–0·052	Severe	Weak
CaCl$_2$.2H$_2$O	0·013–0·034	None	Good
CaSO$_4$	0·0–0·031	Severe	Absent
BaCl$_2$.2H$_2$O	0·022–0·052	Severe	Absent

Plate 4 (1) shows a section under polarized light of a seam from a cheese into which MgSO$_4$ had been incorporated. Crystals are seen concentrated along the seam and fusion was weak. Plate 4 (2), showing a section through a seam of a cheese made with CaCl$_2$, demonstrates that there is no concentration of crystals along the seam. Fusion was complete.

Effect of insoluble material on fusion

Curd particles with sand grains on the surfaces did not fuse as well as particles of uncontaminated curd.

Identity of crystals in the seams

The X-ray patterns showed that the crystalline material in the seams of commercially-made salted cheese was calcium orthophosphate, CaHPO$_4$.2H$_2$O. The material adjacent to the seams gave a similar but very weak X-ray pattern. Microscopic examination showed that the seam contained fine-grained crystals with a mean refractive index between 1·550 and 1·540, consistent with that of calcium orthophosphate dihydrate.

The crystals in the seams of curd salted with MgSO$_4$ were shown by X-ray techniques to be CaSO$_4$.2H$_2$O.

The release of moisture and of calcium and phosphate ions from cheddared curd in the presence of various amounts of salt

Fig. 1 shows that there is a linear relationship between the amounts of moisture, of calcium and of phosphate ions expelled from slices of cheddared curd and the amount of salt added up to about $2\frac{1}{2}$%. A departure from linearity above this level was no doubt due to the fact that some salt remained undissolved at the end of the test period of 70 min.
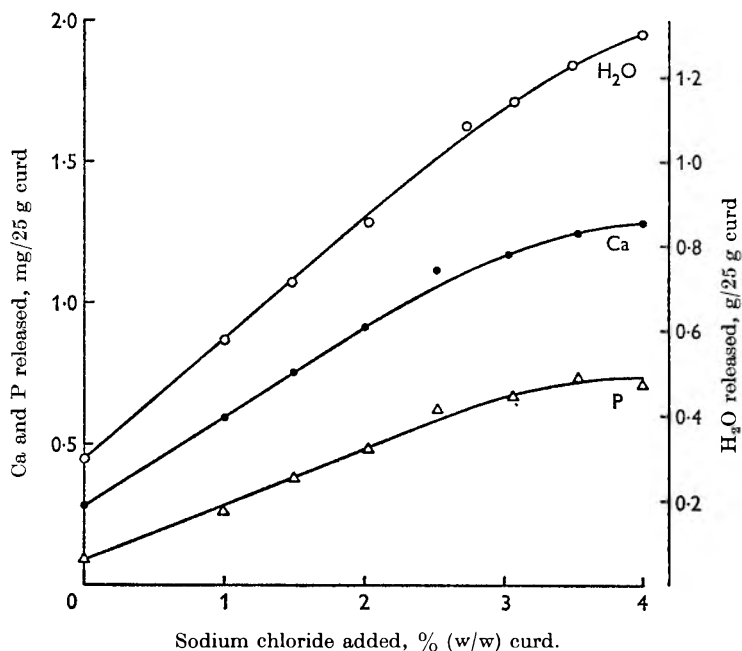
Fig. 1. The release of moisture and of calcium and phosphate ions from cheddared curd in the presence of various amounts of salt.
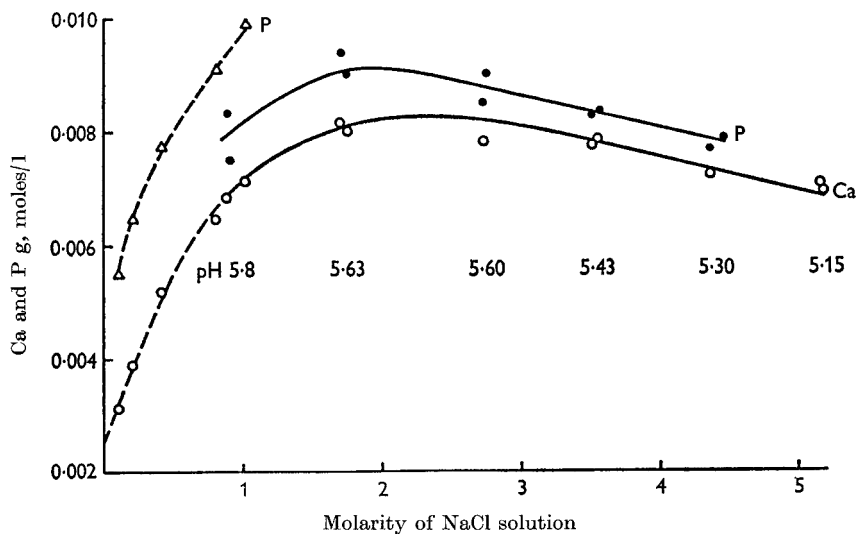
Fig. 2. The solubility of $CaHPO_4 . 2H_2O$ in salt solutions. ●, Phosphorus content; ○, Ca content; – – –, from results of Lugg (1931).

The effect of curd pH and of the addition of sodium and calcium chlorides on the release of calcium and phosphate ions

Table 2 shows the effect of sodium chloride on the release of calcium and phosphate ions from cheddared curd of varying acidity. On addition of sodium chloride the

Table 2. *The release of water, calcium and phosphate from 25 g of cheddared curd in the presence and absence of sodium chloride* ($2 \cdot 5 \%$ w/w curd)

Initial curd, pH		Salted			Unsalted			Concentration Ca in H₂O, g moles/l		Concentration P in H₂O, g moles/l		Ratio conc. P/Ca	
		H_2O, g	Ca, mg	P, mg	H_2O, g	Ca, mg	P, mg	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted
5·7	Expt. 1	0·95	0·63	0·35	0·44	0·55	0·21	0·018	0·033	0·013	0·016	1:1·8	1:2·6
	2	0·88	0·65	0·44	0·38	0·52	0·20	0·018	0·035	0·013	0·016	1:1·7	1:2·7
	3	1·39	0·78	0·41	0·62	0·99	0·32	0·015	0·040	0·010	0·016	1:1·9	1:3·1
5·5	Expt. 1	1·16	0·87	0·46	0·43	0·64	0·23	0·020	0·038	0·013	0·016	1:2·0	1:3·0
	2	1·13	0·98	0·70	0·38	0·77	0·28	0·023	0·050	0·019	0·023	1:1·4	1:2·7
	3	1·40	0·88	0·51	0·61	1·14	0·37	0·015	0·043	0·013	0·019	1:1·8	1:2·8
5·4	Expt. 1	1·16	1·20	0·62	0·50	1·15	0·38	0·025	0·058	0·016	0·026	1:2·0	1:2·9
	2	1·25	1·27	1·03	0·40	0·78	0·36	0·025	0·050	0·026	0·029	1:1·2	1:2·2
	3	1·51	1·29	0·79	0·59	1·30	0·47	0·023	0·055	0·016	0·026	1:1·6	1:2·3

The curd moistures were: expt. 1, 40·8 %; expt. 2, 39·2 %; expt. 3, 38·7 %.

Table 3. *The release of water, calcium and phosphate from 25 g of cheddared curd in the presence and absence of calcium chloride* ($3 \cdot 1 \%$ w/w curd)

Curd pH		Salted			Unsalted			Ratio Ca/H₂O as g moles/l		Ratio P/H₂O as g moles/l		Ratio conc. P/Ca	
Initial	Final	H_2O, g	Ca, mg	P, mg	H_2O, g	Ca, mg	P, mg	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted
5·5	5·25	1·36	—	0·06	0·52	1·00	0·21	—	0·048	0·0013	0·013	—	1:1·21
5·4	5·16	1·56	—	0·21	0·64	1·64	0·38	—	0·098	0·0042	0·019	—	1:1·5
5·3	5·10	1·61	—	0·18	0·63	1·70	0·46	—	0·093	0·0035	0·023	—	1:1·9

release of water and of calcium and phosphate ions from salted curd was greater than from unsalted curd and the lower the pH the greater was the amount of these ions released. The effect of pH was evident with both salted and unsalted curd. It will be seen from Table 3 that when calcium chloride was added, despite the marked drop in pH, the release of phosphate was suppressed in contrast to a significant increase due to the addition of sodium chloride. Ter Horst (1963) found that when calcium chloride was added to milk the amount of both Ca^{++} and $CaPO_4'$ bound to the casein was greatly increased. In both our experiments there was a substantial increase in the release of phosphate with a decrease in the initial pH of both treated and untreated curd.

The solubility of calcium orthophosphate dihydrate in salt solutions

The results of the analyses for Ca^{++} and PO_4''' in salt solutions of strength 0·8–5·2 M together with values given by Lugg (1931) for 0·1–1·0 M salt solutions are shown in Fig. 2. Lugg's results are for 40 °C, those for the present study for 20 °C.

The values indicate that the solubility of the orthophosphate rose steeply from about 0·0025 M in water to a maximum of about 0·008 M in a 2 M sodium chloride solution. With further increase in the concentration of sodium chloride to 5·2 M the solubility of the orthophosphate declined to approximately 0·007 M. Between 0·8 and 5·2 M sodium chloride the pH of the solutions declined from 5·80 to 5·15.

<div style="text-align:center">DISCUSSION</div>

The nature of seaminess

The results from microanalyses and from the microscopical examination of sections of seamy cheese demonstrate that the seams are due not to a layer of fat on the surface of the curd particles but to a layer of crystals of $CaHPO_4.2H_2O$. The photomicrographs show further that in both seamy and non-seamy Cheddar cheese, crystals are scattered throughout the body, but in seamy cheese they are concentrated in addition in the vicinity of the surfaces of the milled curd particles to which sodium chloride was applied. To a depth of about 20 μm below these surfaces the protein appears to be denser than elsewhere and the zones immediately behind the contracted curd are depleted of crystals. Our results differ from those of Swiatek & Jaworski (1959) who demonstrated crystals throughout the body of Roquefort, Swiss, Tilsit and Trappist cheese and established that the main component was calcium orthophosphate of unspecified form; they did not find any crystal aggregation between the surfaces of fusion of the curd particles. This difference is probably due to the cheeses they examined being brine salted.

Curd fusion

In considering the reasons for poor fusion of the curd particles it is of interest to note that Adachi (1963) showed that the size of the micelles formed by the interaction of sodium caseinate and alkaline earth metal chlorides varied with the nature of the cation; magnesium and barium gave the smallest micelles and calcium the largest. It is possible that the same forces which determine the size of the micelles may also influence the degree of fusion of the curd. This would account for the good fusion in the presence of calcium chloride and poor fusion with magnesium and barium chlorides.

Poor fusion of the curd as a consequence of heavy salting will also result from irreversible changes in the protein at the surface, from poor contact between the hardened surfaces, from the physical separation brought about by the presence of salt crystals and, when these have disappeared, from the growth of the calcium orthophosphate crystals.

The cause of seaminess

In the manufacture of Cheddar cheese the slabs of curd after cheddaring are cut into pieces about $\frac{1}{2}$ in. square in cross-section and 2–3 in. long. Salt is then added at a rate of 2–3% of the weight of the curd to give a final salt content in the cheese of 1·5–1·8%.

The salt causes the curd to contract and expel whey containing fat, lactose and mineral salts, including some of the added sodium chloride. At the same time the surfaces of the curd particles become firm and tough.

About 30–40 min after the salt is added, the curd is pressed into moulds and held under pressure for at least 6 h, but usually overnight, by which time the particles will have fused together. Curd with a moisture content below 36% treated with $2\frac{1}{2}$–3% of salt is very likely to develop seaminess.

The rate of penetration of salt into cheese curd is very slow, about 0·05 in. in 15 min and 0·15 in. in 75 min (McDowall & Dolby, 1936). Thus the concentration of salt in the liquid on the surface of the curd particle remains high for some hours after the salt is added. It will probably require some weeks for the final equilibrium salt concentration of about 0·8 M to be established in the serum of the cheese.

The present study provides evidence on which an understanding of the mechanism of seam formation may be based. Prior to the pressing of the curd the initial high concentration of sodium chloride on the surface of the curd particles causes water (whey) and with it Ca and P ions to be drawn to the surfaces of the particles from which some of it will drain away. The results show that this whey is more dilute with respect to Ca and P than is whey from unsalted curd. Furthermore, there is an increase in the ratio of P to Ca under the influence of salt. These phenomena were also observed by McDowall & Dolby (1935, 1936) who attributed this effect to an osmotic diffusion of water from the curd, the salted surface acting as a selectively permeable membrane.

After pressing, the salted particles do not fuse for some hours but drainage of the whey from the surfaces is arrested. Thus a film of water with various ions in solution and some still undissolved sodium chloride crystals separate the unfused adjoining curd particles. It can be seen from the values for the solubility of $CaHPO_4.2H_2O$ and the composition of the whey that the concentrations of calcium and phosphate ions in this film of liquid exceed the solubility of $CaHPO_4.2H_2O$ in the equilibrium salt solution of cheese (about 0·6–0·8 M). Because of the strong influence of sodium chloride concentration on the solubility of $CaHPO_4.2H_2O$ it is possible to attribute the crystallization of the compound to diffusion of the sodium chloride away from the seam. Migration of calcium and phosphate ions back into the curd particles, along with the trapped water and sodium chloride, may also be countered by the displacement of more calcium and phosphate ions from the curd by the sodium chloride; such displacement is to be expected and that it takes place is supported by

the X-ray studies which showed the presence of phosphate crystals throughout the body of the cheese. In addition, the hypothesis of McDowall & Dolby (1935, 1936) might be extended by postulating that the curd surfaces on prolonged contact with sodium chloride become more selective and impede the return of the calcium and phosphate ions.

Other factors which would favour crystallization are the fall in temperature of the cheese which occurs during and after pressing and physical forces arising from movement between the curd surfaces during pressing.

The control of seaminess

In the commercial production of Cheddar cheese, beside sthe moisture content of the curd and the level of salting, the size of the milled curd particles influences the occurrence of seaminess. The larger the curd particles the smaller the surface to volume ratio and therefore the higher the surface salting rate for a given final content in the cheese.

The observation of Czulak (1963) that seaminess is reduced by washing of the curd after milling and before salting can be explained by the removal of phosphate from the surface layer. In addition, the provision of more water by reducing the concentration of salt on the surface of the curd particle will lessen the dehydrating and contracting effect of the surface layer and speed up the diffusion of salt.

Comparison of the amounts of phosphate released from salted and unsalted curd at the 3 pH levels indicates that the lower the pH of the curd the greater is the amount of phosphate released. This suggests the possibility of reducing seaminess by applying the salt at as low a pH as is otherwise consistent with the production of good quality cheese, and thus allowing the released phosphate to escape before the curd is pressed.

The experiments showing the effect of calcium chloride on the release of phosphate suggest the possibility of controlling seaminess by washing the curd particles with a solution of calcium chloride to suppress the release of phosphate prior to addition of the sodium chloride.

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EXPLANATION OF PLATES

PLATE 1

Seamy cheese (natural size).

PLATE 2

1. Seam of cheese under ultra-violet light showing the less porous regions along the seam.

2. Seam of cheese under ultra-violet light showing an open section.

PLATE 3

1. Non seamy cheese under polarized light.

2. Seamy cheese (commercial) under polarized light.

PLATE 4

1. Seam of cheese made with $MgSO_4$ under polarized light.

2. Seam of cheese made with $CaCl_2$ under polarized light.

Seam

Seam

1

2

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Plate 3

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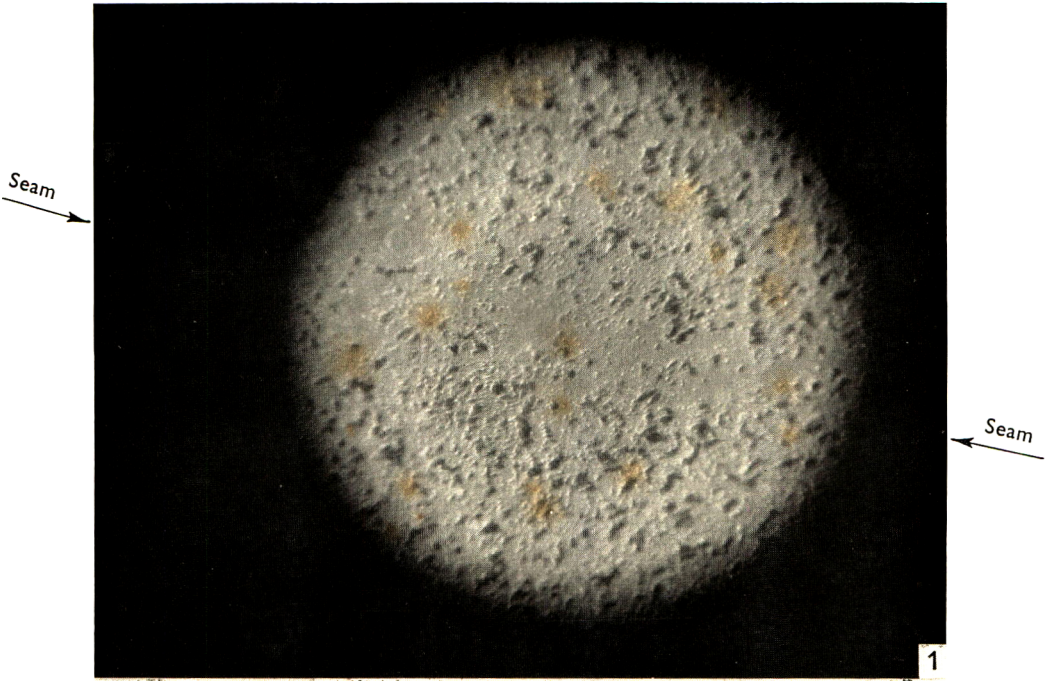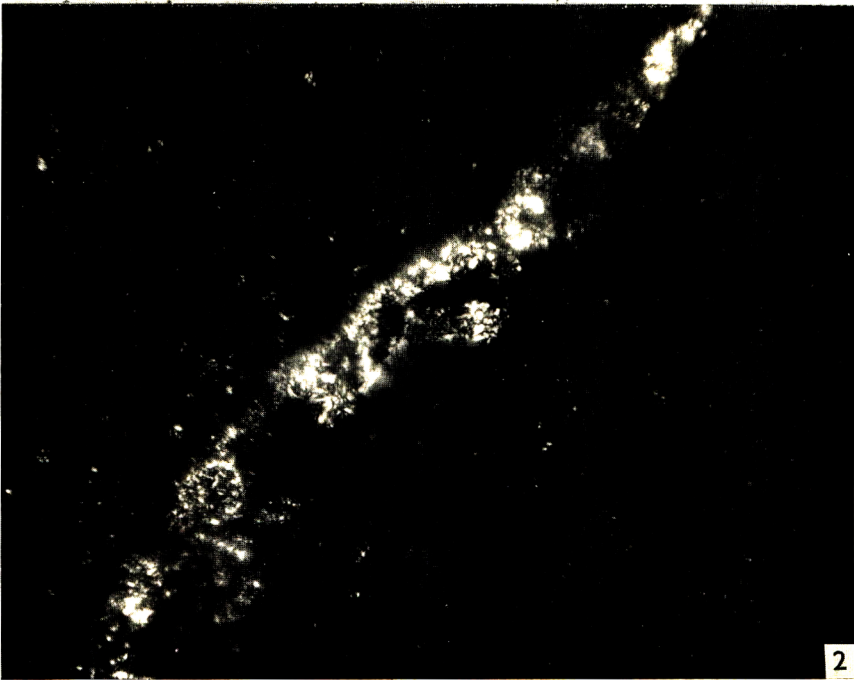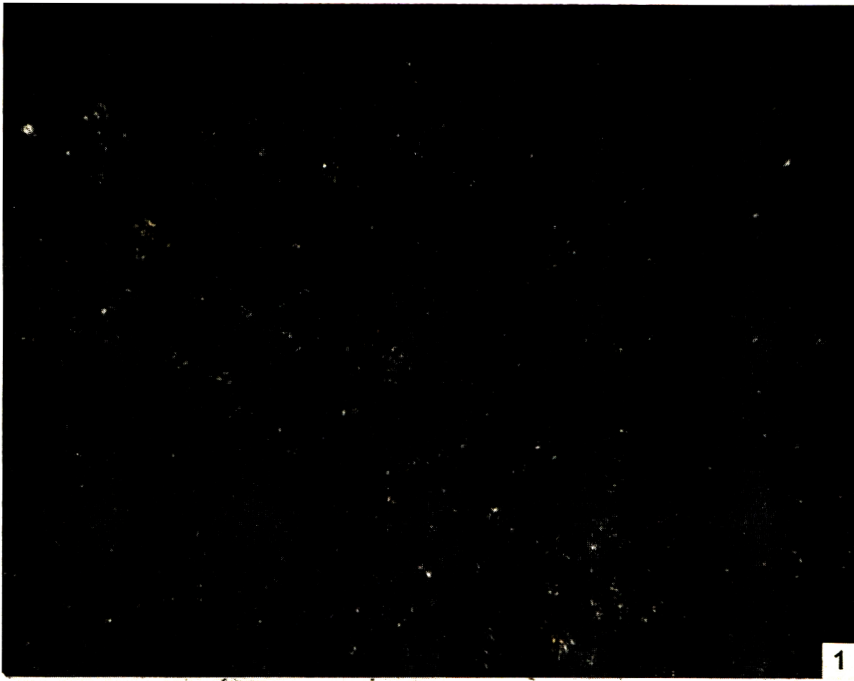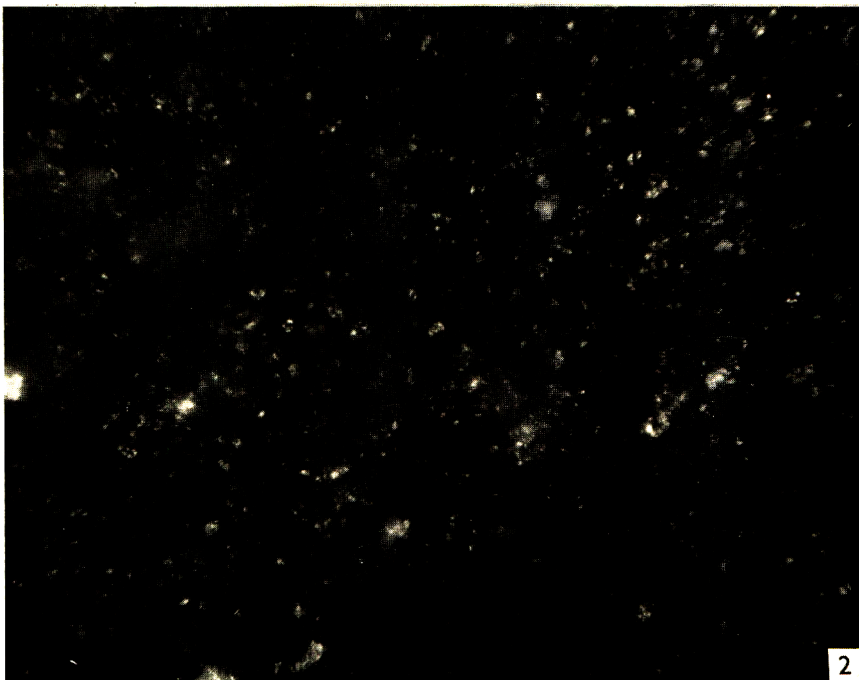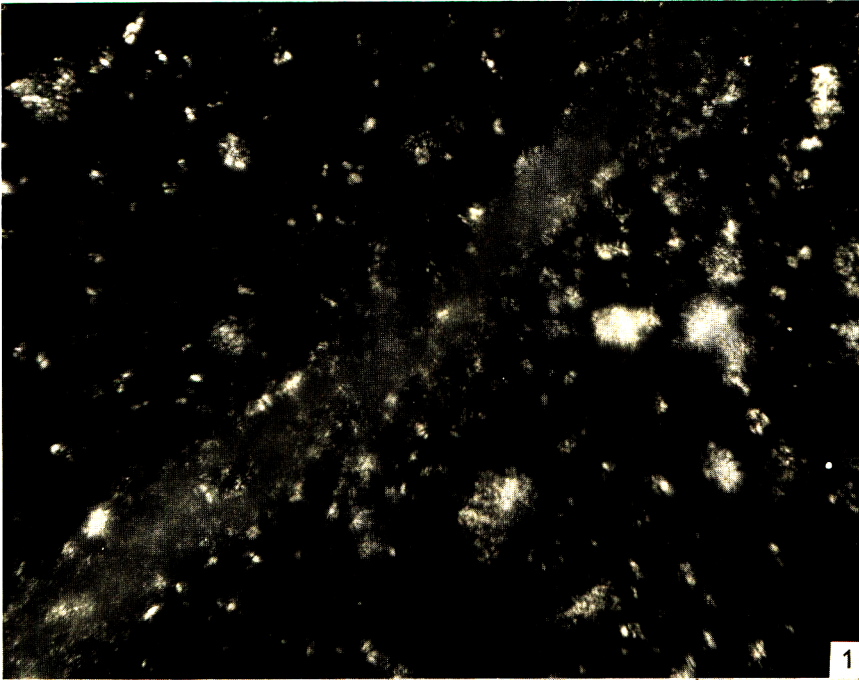
Effect of stage and number of lactation on the yield and composition of cow's milk

By J. A. F. ROOK and R. C. CAMPLING

National Institute for Research in Dairying, Shinfield, Reading

SUMMARY. The effects of stage of lactation and of age on milk and composition yield of cows kept under controlled conditions of feeding and management were investigated. Two groups each of 3 Friesian heifers were used: one group (*A*) was given a daily diet of 18 lb hay and concentrates at the rate of 3·8 lb/10 lb of milk and kept on experiment throughout the first 3 lactations; a second group (*B*) was given the same amount of hay and a constant daily amount of concentrates of 10·7 lb and was kept on experiment throughout the 1st lactation only.

The total yields of milk and peak yields for the 1st lactation were similar in both groups. In all the cows, fat content was high early and late in lactation and varied little throughout the mid-lactation period. With cows of group *A*, the content of solids-not-fat declined until about the 5th week of lactation and then remained steady until a marked rise occurred late in lactation. With cows of group *B*, however, the content of solids-not-fat was at a minimum between the 5th and 10th weeks of lactation and then increased progressively until late lactation when a more marked rise occurred. Lactose content increased rapidly in the first few days of lactation and then remained constant for several months, eventually declining late in lactation. Characteristic changes were also observed in the contents of total protein, and of the various protein fractions, and of the major minerals of milk.

The concentrations of all major constituents in milk secreted from the 2nd–5th months of lactation tended to decrease from lactation to lactation.

The results are discussed in relation to the development of knowledge of the changes in milk composition throughout lactation.

The main trends in milk composition associated with stage of lactation and age have been established in several investigations (see Rook, 1961). Little attempt has been made, however, to distinguish between effects due to changes in the physiological activity of an animal throughout lactation or with age, and those due to changes in level and type of feeding or incidence of udder damage linked with stage of lactation and age. In the investigation reported here, the effects of stage of lactation and age on milk yield and composition were determined in a group of animals maintained throughout indoors under carefully controlled conditions of feeding and management. Variations with season due to the associated changes in nutrition that are common under farm conditions and to clinical infections of the udder, were thus largely excluded.

Animals and experimental design

Six Friesian heifers due to calve over a period of about 1 month were taken from the Institute herd. The animals were brought in from grass during the 7th week before the expected date of calving and housed separately in standings in the farm cowshed. At calving, the animals were allocated alternately to one of 2 feeding treatments, *A* or *B*. Animals on treatment *A* were kept on experiment until the completion of their 3rd lactation but animals on treatment *B* were removed from experiment at the end of their 1st lactation.

Management

Calves were removed from their dams at birth and were not permitted to suckle. The dams were milked immediately after parturition, 6 h later and then at normal milking times either until the 333rd day of lactation or until the daily milk yield had dropped to less than 10 lb. The animals were then milked once daily for 7 days and dried off. They were served at about the 16th week after calving to give an interval between calvings of 400 days.

Feeding

Throughout the whole of the experiment a standard hay allowance of 18 lb was offered daily to each cow on experiment. During the last 6 weeks before the expected date of calving the animals were offered additionally a dairy concentrate cube at a daily rate of 2 lb/cow for the first 2 weeks, 4 lb for the next 2 weeks and then 6 lb until calving. After calving the allowance of concentrates was as follows:

Treatment A. Concentrates were offered daily at a rate of 14·2 lb/cow for the 1st week and 14·5 lb for the 2nd and 3rd weeks after calving. Thereafter, the concentrate allowance was adjusted weekly, on the basis of the yield of milk in the previous week, to a rate of 3·8 lb/10 lb milk.

Treatment B. Concentrates were offered at a daily rate of 10·7 lb/cow throughout lactation. For an animal yielding 9500 lb in a lactation lasting 340 days, the total amounts of concentrates offered under treatments *A* and *B* would be the same.

Refused portions of food were collected daily and weighed. A fresh batch of hay was acquired each season to meet the requirements for the whole of the next lactation. The dairy concentrate cube was prepared from a mixture of barley 17 parts, maize 20, wheat bran 20, decorticated groundnut meal 15, copra cake 10, palm kernel cake 5, molasses 10, dicalcium phosphate 1, calcium carbonate 1, and salt 1 % with 5×10^6 i.u. vitamin A and 1×10^6 i.u. vitamin D added per ton.

Sampling and methods of analysis

Milk yield was recorded at each milking. Samples of milk were taken at each milking during the 1st lactation and at regular but less frequent intervals throughout the 2nd and 3rd lactations. Weighted, composite samples representing 3- or 4-day periods were prepared for each cow and analysed for fat and total solids, and in addition certain of the samples were analysed for lactose, total N and N distribution, sodium, potassium, calcium and phosphorus. The frequency with which detailed

analyses were made is indicated in the figures and tables. The methods of analysis for fat, total solids, lactose and total N and N distribution have been described previously (Rook & Line, 1961). Sodium and potassium were determined by the methods described by Barry & Rowland (1953). Calcium and phosphorus were determined by volumetric procedures after the separation of calcium as the oxalate and of phosphorus as the phosphomolybdate from a solution of milk ash.

<div align="center">RESULTS</div>

<div align="center">*Variations in yield and composition of milk throughout the 1st lactation*</div>

Food intake (Table 1)

Small amounts of hay were refused by all the cows from time to time but, with the exception of Maid 8 who refused on average 2·1 lb/day, the mean daily refusals were negligible. There was no refusal of concentrates. The yield of milk in the 1st lactation was, in all animals, considerably less than the assumed value used in the calculation of the concentrate allowance for treatment *B*, and as a result the mean intake of concentrates per lb of milk produced was higher in cows receiving treatment *B*. This is reflected in the higher gain in body-weight in group *B* (Fig. 1).

Table 1. *The length of the lactation, the lactation yield, and the mean values for the intakes of hay and concentrates for the 6 heifers in their 1st lactation*

Cow	Treatment*	Length of lactation, days	Lactation yield, lb	Mean daily intake of hay, lb	Mean intake of concentrates/lb of milk produced, lb
Bugle 4	*A*	326	6,592	17·9	0·41
Germander 4		327	8,391	17·7	0·40
Grace 3		319	7,433	17·8	0·40
Gaiety 2	*B*	345	8,124	17·6	0·46
Grouse 3		343	8·732	17·9	0·42
Maid 8		307	6,155	15·9	0·53

<div align="center">* See p. 46 for details.</div>

Milk yield (Fig. 1)

The total lactation yields and the peak yields in the 2 groups of animals were similar but animals in group *B* showed the flatter lactation curve from the 4th month of lactation until the marked, late lactation decline.

Fat content (Figs. 1 and 2)

The well-established pattern of a decrease in fat content at the beginning of lactation and an increase towards the end was observed in all the cows. Small variations in fat content occurred from the 3rd–8th month of lactation but were not consistent from cow to cow in either treatment group.

Solids-not-fat, protein and lactose contents (Figs. 1, 2, 3 and 4)

High levels of solids-not-fat (SNF) were observed in the colostrum in all cows but the content varied widely from cow to cow (10·6–26·6%). The content of SNF declined rapidly at first and then more slowly, to about the 5th week of lactation. In

animals in group A, the levels of SNF then remained steady until the typical increase occurred late in lactation in association with a sharp decline in milk yield. In animals of group B, the SNF content was at a minimum between the 5th and 10th weeks of
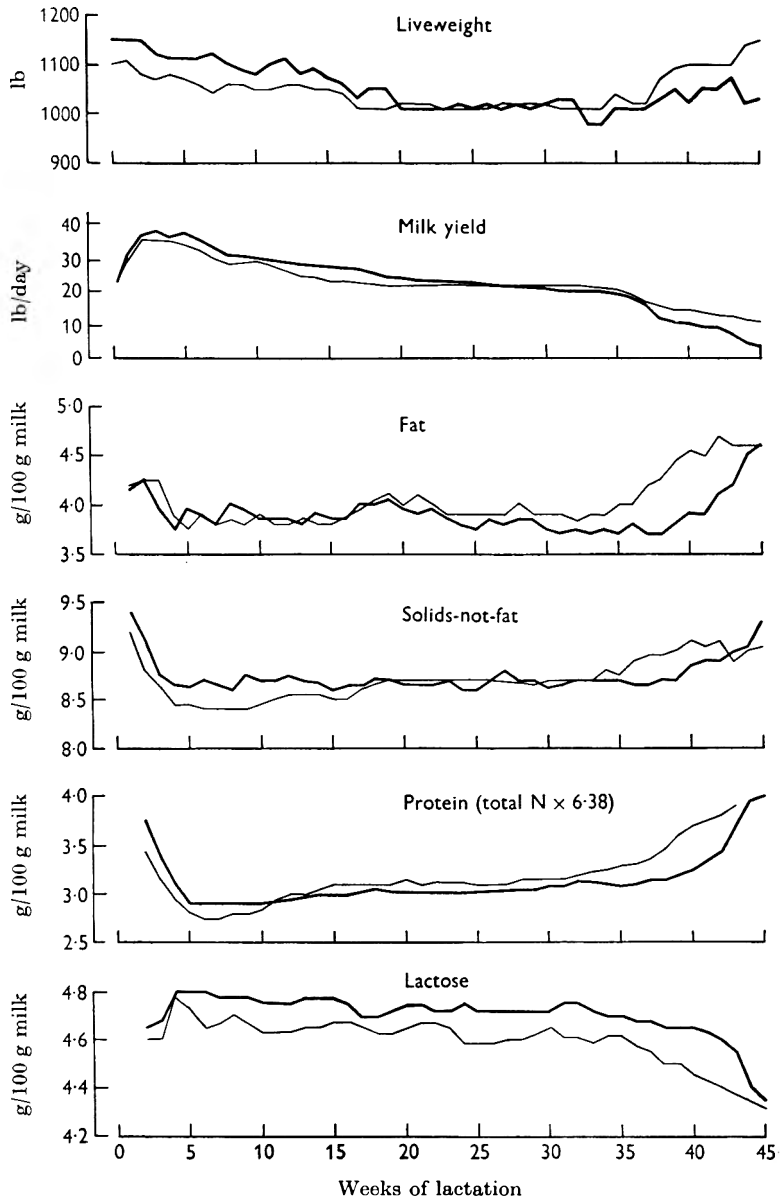
Fig. 1. Variations throughout the 1st lactation in liveweight, milk yield and fat, SNF, lactose (anhydrous) and protein (total N × 6·38) contents of milk. —, Mean values for cows on treatment A; —, mean values for cows on treatment B.

lactation and increased progressively throughout the mid-lactation period until the sharp increase during late lactation. The difference between the groups in SNF content in the mid-lactation period was mainly due to a difference in protein content.

In both groups, the total protein content was high in colostrum but then decreased rapidly to a minimum between the 5th and 10th weeks of lactation. Thereafter, the content increased slightly but then remained steady from about the 15th week until a sharp increase occurred late in lactation. The early lactation minimum was more pronounced in animals in group *B* than in those in group *A*. The changes in casein content were closely similar to those for total protein content, except during the first few days of lactation when the contents of globulin and proteoses were exceptionally high. The residual albumin contents also followed a pattern similar to that of casein
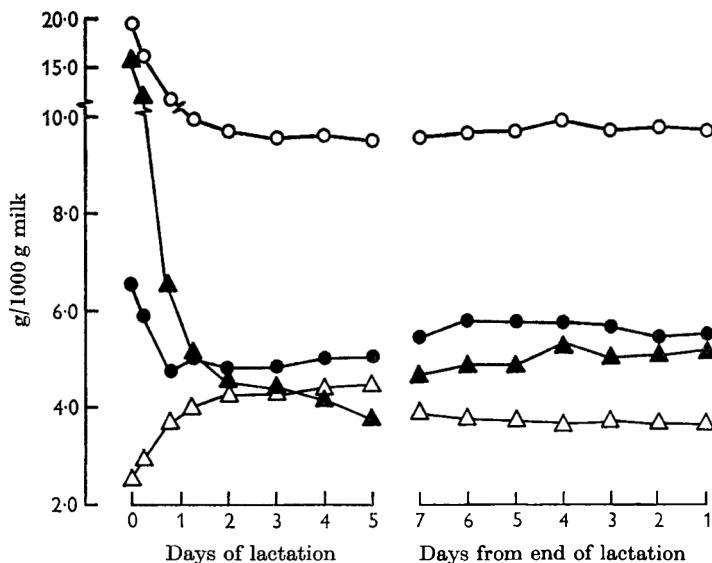
Fig. 2. Variations throughout the 1st 5 and the last 7 days of the 1st lactation in fat, SNF, lactose (anhydrous) and protein (total N × 6·38) contents of milk. Values are the mean for 6 cows. ●, Fat; ○, SNF; △, lactose; ▲, protein.

but, from about the 14th week of lactation, the pattern for β-lactoglobulin was quite distinct; its content rose to a maximum in mid-lactation and then declined progressively and, except in one cow, showed no increase late in lactation. Proteoses and globulins were major proteins of colostrum but the concentrations fell rapidly, over a period of about 5 days, to very low levels and remained low until about the 40th week of lactation, when they increased to the end of lactation. The concentrations of proteoses (1·00–2·96 %) and of globulins (0·32–11·99 %) in the first colostrum varied markedly from cow to cow, and this variation accounted for much of the differences between cows in the SNF content of colostrum. In 5 out of the 6 cows, globulins were present in the first colostrum in higher concentration than were proteoses. In Bugle 4, however, the reverse was true and the globulin content (0·32 %) of the colostrum was only slightly above that of normal milk: this may have been related to a delay in calving of about 14 days past the expected calving date, during which period the udder was grossly distended and milk leaked from the teats. The non-protein N content was high in colostrum and, to a lesser extent, in milk secreted very late in lactation but showed little variation throughout the major part of the lactation.

4

The content of lactose increased sharply in the first few days of lactation but then remained remarkably constant for several months, and in some cows until the 8th or 9th month of lactation, after which the level declined.
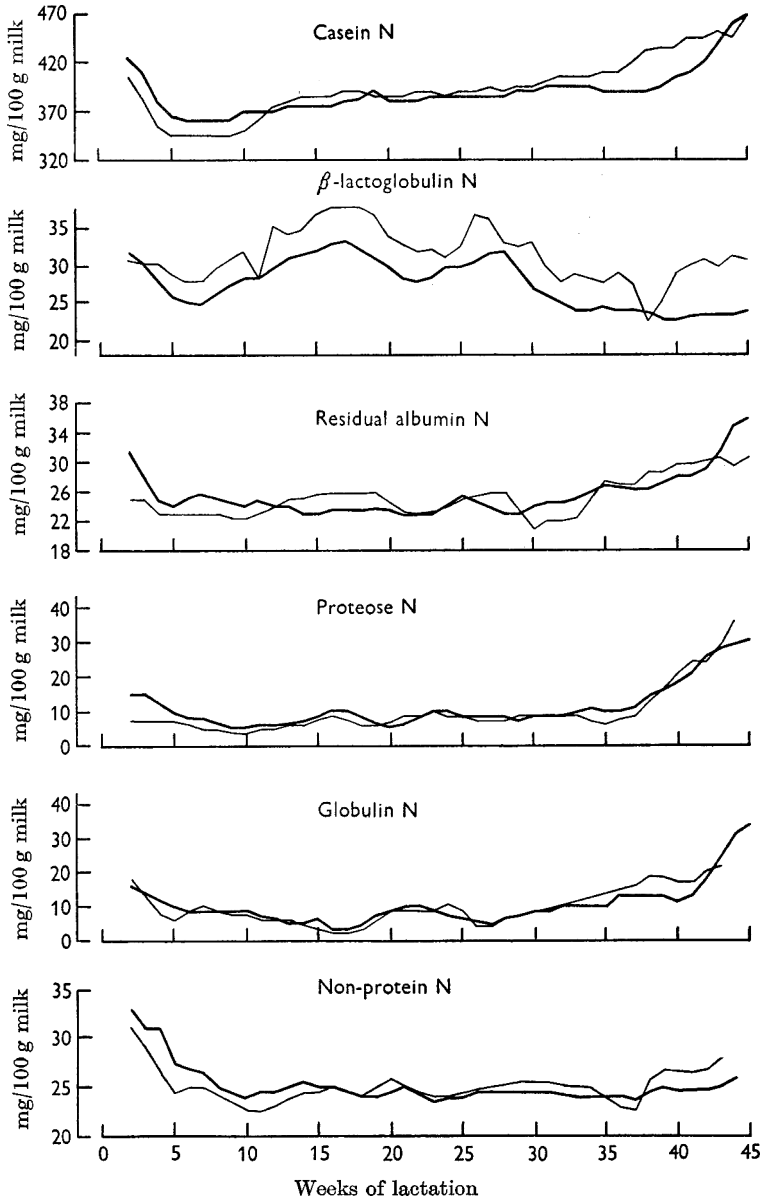
Fig. 3. Variations throughout the 1st lactation in the contents in milk of the various nitrogenous fractions. ━, Mean values for cows on treatment A; —, mean values for cows on treatment B.

Sodium, potassium, calcium and phosphorus contents (Figs. 5 and 6)

Comparatively high concentrations for sodium were observed in colostrum, more particularly in samples from Bugle 4 and Gaiety 2 which were contaminated with blood. Sodium content was fairly steady in early and mid-lactation but increased noticeably towards the end of lactation. Variations in potassium content in the 1st and 2nd days of lactation were not consistent from cow to cow, but in all the cows,
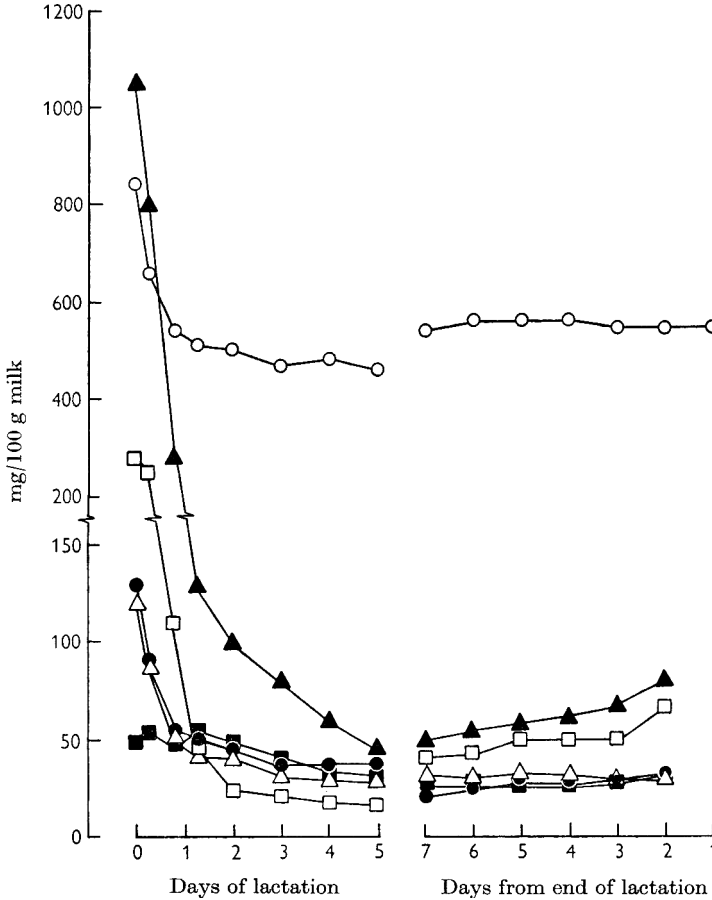
Fig. 4. Variations throughout the 1st 5 and the last 7 days of the 1st lactation in the contents in milk of the various nitrogenous fractions. Values are the mean for 6 cows. ○, Casein N; ●, β-lactoglobulin N; △, residual albumin N; □, proteose N; ▲, globulin N; ■, non-protein N.

from about the 3rd day of lactation onwards, the content varied little until a decline occurred late in lactation. Concentrations of calcium and of phosphorus were high in colostrum with the exception of the values for Bugle 4 of 101 mg Ca/100 g and 83 mg P/100 g. Subsequently, the concentrations declined rapidly and then remained comparatively steady until the values increased again late in lactation.

Variations in yield and composition of milk with age (Table 2)

The concentrations of the major constituents in milk secreted during the period from the 5th–22nd week of lactation tended to decrease from lactation to lactation,
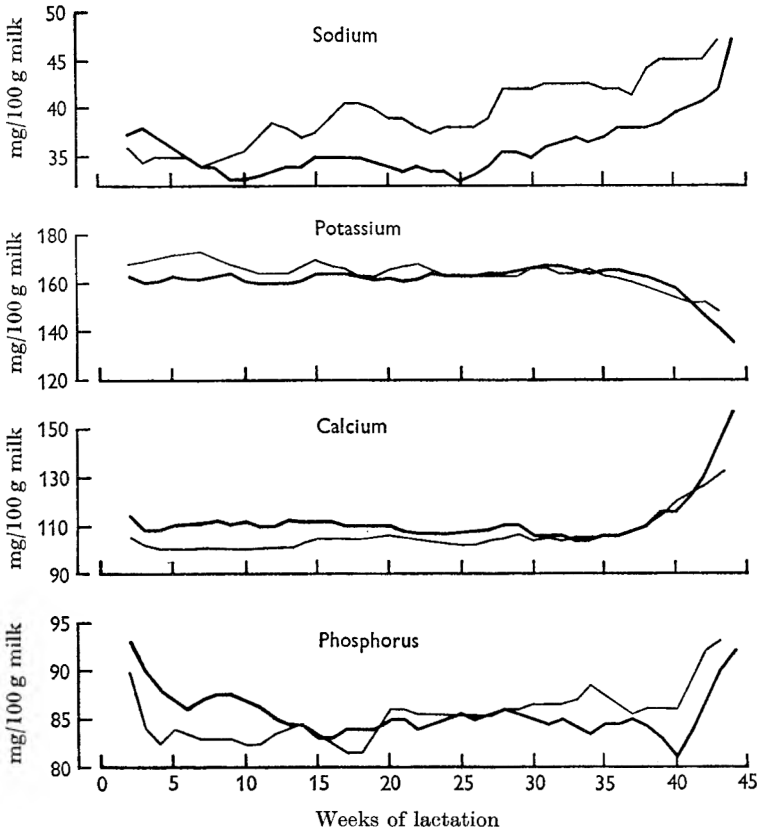
Fig. 5. Variations throughout the 1st lactation in sodium, potassium, calcium and phosphorus contents of milk. —, Mean values for cows on treatment A; —, mean values for cows on treatment B.
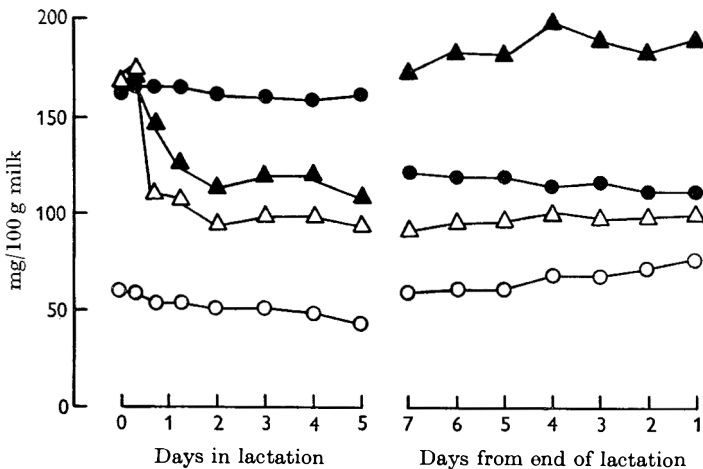
Fig. 6. Variations throughout the 1st 5 and the last 7 days of the 1st lactation in the sodium, potassium, calcium and phosphorus contents of milk. Values are the mean for 6 cows. ○, Sodium; ●, potassium; △, calcium; ▲, phosphorus.

and the changes were accompanied by small increases in the concentrations of proteoses plus globulins and of the residual albumin fraction, which includes serum albumin.

Table 2. *Mean values, with standard error, for the concentrations of major constituents of milk for the 3 cows on treatment A in the 1st, 2nd and 3rd lactations*

(Values were calculated for the period from the 5th to 22nd week of lactation. The figures in parentheses are the numbers of samples on which the values are based.)

Lactation no. ...	1	2	3
		Bugle 4	
Fat, %	$4\cdot34\pm0\cdot021$ (36)	$4\cdot21\pm0\cdot042$ (18)	$4\cdot04\pm0\cdot080$ (8)
Solids-not-fat, %	$8\cdot51\pm0\cdot012$ (36)	$8\cdot43\pm0\cdot022$ (18)	$8\cdot34\pm0\cdot048$ (8)
Lactose, anhydrous, %	$4\cdot72\pm0\cdot026$ (9)	$4\cdot64\pm0\cdot042$ (5)	$4\cdot63\pm0\cdot049$ (4)
Total N, mg/100 g	$462\pm4\cdot8$ (9)	$471\pm7\cdot5$ (5)	$449\pm9\cdot6$ (3)
Casein N, mg/100 g	$365\pm4\cdot1$ (9)	$374\pm5\cdot2$ (5)	$352\pm12\cdot0$ (2)
β-lactoglobulin N, mg/100 g	$30\cdot3\pm1\cdot41$ (9)	$30\cdot6\pm2\cdot07$ (5)	$26\cdot0\pm2\cdot01$ (2)
Residual albumin N, mg/100 g	$23\cdot8\pm0\cdot53$ (9)	$23\cdot4\pm0\cdot78$ (5)	$25\cdot5\pm1\cdot58$ (2)
Proteose + globulin N, mg/100 g	$15\cdot9\pm1\cdot61$ (9)	$20\cdot2\pm1\cdot07$ (5)	$17\cdot5\pm6\cdot09$ (2)
Non-protein N, mg/100 mg	$26\cdot9\pm0\cdot46$ (9)	$22\cdot4\pm0\cdot71$ (5)	$23\cdot5\pm1\cdot58$ (2)
		Germander	
Fat, %	$3\cdot92\pm0\cdot027$ (35)	$3\cdot72\pm0\cdot033$ (17)	$3\cdot50\pm0\cdot117$ (8)
Solids-not-fat, %	$8\cdot79\pm0\cdot011$ (35)	$8\cdot61\pm0\cdot017$ (17)	$8\cdot63\pm0\cdot045$ (8)
Lactose, anhydrous, %	$4\cdot75\pm0\cdot021$ (9)	$4\cdot75\pm0\cdot038$ (4)	$4\cdot75\pm0\cdot033$ (4)
Total N, mg/100 g	$477\pm3\cdot2$ (9)	$458\pm3\cdot6$ (4)	$448\pm5\cdot0$ (3)
Casein N, mg/100 g	$383\pm3\cdot2$ (9)	$370\pm3\cdot1$ (4)	$361\pm3\cdot5$ (2)
β-lactoglobulin N, mg/100 g	$30\cdot2\pm1\cdot15$ (9)	$25\cdot3\pm1\cdot50$ (4)	$18\cdot5\pm0\cdot71$ (2)
Residual albumin N, mg/100 g	$24\cdot2\pm0\cdot65$ (9)	$24\cdot8\pm0\cdot71$ (4)	$27\cdot5\pm3\cdot50$ (2)
Proteose + globulin N, mg/100 g	$15\cdot2\pm0\cdot83$ (9)	$15\cdot5\pm0\cdot65$ (4)	$21\cdot0\pm0\cdot00$ (2)
Non-protein N, mg/100 mg	$23\cdot8\pm0\cdot41$ (9)	$22\cdot3\pm1\cdot26$ (4)	$23\cdot5\pm0\cdot71$ (2)
		Grace 3	
Fat, %	$3\cdot48\pm0\cdot021$ (37)	$3\cdot56\pm0\cdot035$ (17)	$3\cdot48\pm0\cdot072$ (8)
Solids-not-fat, %	$8\cdot67\pm0\cdot006$ (37)	$8\cdot56\pm0\cdot021$ (17)	$8\cdot44\pm0\cdot025$ (8)
Lactose, anhydrous, %	$4\cdot77\pm0\cdot020$ (10)	$4\cdot82\pm0\cdot006$ (4)	$4\cdot69\pm0\cdot027$ (4)
Total N, mg/100 g	$461\pm2\cdot8$ (10)	$454\pm6\cdot1$ (4)	$436\pm4\cdot88$ (4)
Casein N, mg/100 g	$370\pm3\cdot2$ (10)	$369\pm4\cdot9$ (4)	$348\pm5\cdot2$ (4)
β-lactoglobulin N, mg/100 g	$28\cdot1\pm0\cdot93$ (10)	$25\cdot3\pm1\cdot26$ (4)	$20\cdot3\pm1\cdot46$ (4)
Residual albumin N, mg/100 g	$24\cdot6\pm0\cdot38$ (10)	$22\cdot5\pm0\cdot65$ (4)	$25\cdot3\pm1\cdot28$ (4)
Proteose + globulin N, mg/100 g	$14\cdot7\pm1\cdot15$ (10)	$14\cdot3\pm1\cdot33$ (4)	$19\cdot3\pm1\cdot06$ (4)
Non-protein N, mg/100 mg	$24\cdot2\pm1\cdot40$ (10)	$23\cdot3\pm0\cdot87$ (4)	$24\cdot3\pm0\cdot62$ (4)

DISCUSSION

It is of interest to recall that Richmond (1899), in the first edition of his textbook on *Dairy Chemistry*, described in some detail, on the basis of published evidence, the high concentrations of casein, soluble proteins and ash and the low concentration of lactose in colostrum, and how composition altered in the first days and weeks of lactation as colostrum changed to normal milk. Of other effects of stage of lactation

he stated: 'Generally speaking, the milk of newly-calved cows is poorer in fat than of cows towards the end of their period of lactation....The casein also increases as the period of lactation advances, while the milk sugar decreases; the mineral matter also increases towards the end of lactation.' These broad conclusions have been substantiated by the many records published since that time of the fat and SNF contents of the milk of cows at different stages of lactation (Ingle, 1902; Crowther, 1905; Speir, 1905; van Slyke, 1908; Crowther & Ruston, 1911; Eckles & Shaw, 1913; Ragsdale & Turner, 1922; Tocher, 1925; Drakeley & White, 1927; Bartlett, 1934; Becker & Arnold, 1935; Bailey, 1952; Provan, 1956). The data have shown invariably high concentrations of fat and SNF at the beginning and end of lactation, but considerable variation in the period of lactation at which the minimum for SNF content, and more particularly for fat content, occurs. Bartlett (1934) and Bailey (1952) have both demonstrated that the marked increase in late lactation in SNF content is observed only in pregnant cows, and that the shape of the lactation curve for SNF is affected by the age of the cow. Information on changes in detailed composition was included in some of the above reports (Crowther & Ruston, 1911, for example), but fuller details have been provided in more recent papers (Azarme, 1938; Bonnier, Hansson & Jarl, 1946; Waite, White & Robertson, 1956; Voigtländer, 1963) and because of improvements in analytical technique the recent results must be accepted as the more reliable.

The most comprehensive investigation so far reported is that of Waite et al. (1956), who analysed samples of milk taken on 6 occasions during the lactation at intervals of approximately 5 weeks, from the end of the 1st month of lactation, from a total of 814 Ayrshire cows. The effects of stage of lactation were separated from those of season and the associated changes in nutrition by statistical means, and the authors concluded: 'Total solids, SNF, fat, crude protein and casein contents fell rapidly for 45 days after calving, with fat and total solids continuing to fall for a further 30 days. The concentrations of all these constituents then increased continuously for the remainder of the lactation, rising more rapidly after about 200 days. The changes in lactose content were opposite from those of fat and protein and smaller in magnitude. The value rose to a maximum after 45 days, fell slowly until about 165 days after calving and then more quickly. The lactose content of milk from cows in their 1st lactation fell much more slowly with advancing lactation than that in the milk of older cows.'

In the present investigation a direct approach to the separation of the effects of stage of lactation from other factors known to affect milk composition was adopted. Our results are broadly in line with the conclusions of Waite et al. (1956) but suggest that when animals are given concentrates according to milk yield only minor changes occur in SNF, total protein, and casein contents in the mid-lactation period. However, there does appear to be a slight, but distinct minimum in protein content between about the 5–10th week of lactation, and this minimum is much more pronounced in animals underfed at peak lactation and overfed late in lactation, as is possibly common in commercial herds. Moreover, under the conditions of the present experiment, lactose content remained remarkably constant throughout the major part of the 1st lactation and, from the limited information available, probably in the 2nd and 3rd lactations also.

Comprehensive studies of the contents of the various milk proteins (Parrish, Wise, Hughes & Atkeson, 1948, 1950) and of minerals (Garrett & Overman, 1940) in colostrum and the milk secreted in the first days of lactation have been reported previously, and our findings are wholly consistent with the published results. Also, the changes in milk composition that occurred from lactation to lactation with the 3 cows in the present investigation maintained on a standard diet under controlled conditions of management were of the same pattern as those reported for commercial herds (Waite *et al.* 1956).

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Studies on the κ-casein complex

II. The isolation of a sialic acid-containing fraction by disrupting the complex at low pH in the presence of sodium chloride

BY R. BEEBY

Division of Dairy Research, C.S.I.R.O., Melbourne, Australia

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SUMMARY. When crude κ-casein was precipitated at pH 3 in the presence of 0·4M-NaCl the supernatant contained up to 80% of the total sialic acid but no detectable cystine or cysteine. Two fractions were obtained from this supernatant by chromatography on DEAE cellulose; one containing 4–6% sialic acid and the other only one-tenth of this amount.

Most of the sialic acid of the sialic acid-rich fraction was soluble in 12% trichloracetic acid following treatment with rennin. It is suggested that the glycopeptide released by the action of the enzyme on casein originates from this fraction.

Evidence has been presented that κ-casein is a complex and that the glycopeptide released from it by rennin stems from one component of this complex (Beeby & Nitschmann, 1963). On the basis of chemical evidence a molecular weight in the region of 50000 was calculated for κ-casein and it was postulated that the complex consists of 3 units each approximately 16000 in molecular weight (Beeby, 1963). It follows that, since all the sialic acid of κ-casein, which is of the order of 2%, is set free during treatment with rennin (Beeby, 1963), the component attacked by the enzyme should contain some 6% of sialic acid.

Beeby & Nitschmann (1963) reported that treatment of κ-casein with urea at pH 4·7 released sialic acid-rich material similar to that set free by low concentrations of rennin. However, these fractions contained 3–4% sialic acid, indicating that they contained 40–50% of one or both of the other components of the complex.

In this paper a method is described for the isolation from κ-casein of a fraction containing up to 6% sialic acid and which appears to be attacked by rennin in the same way as is κ-casein.

EXPERIMENTAL

Materials and methods

All reagents were of analytical grade. Crude κ-casein was obtained from fresh raw bulk milk from a single herd under the conditions recommended by Hill & Hansen (1963) except that 0·25M-CaCl$_2$ was used to split the α-κ-casein complex. Thymol was added to all protein solutions as preservative. De-ionized water from an Elgastat de-ionizing column was used throughout. The Sephadex used was G25 medium grade

from Pharmacia, Sweden and the diethylaminoethyl (DEAE) cellulose was What-man DE 50. Fractions were collected on an LKB fraction-collector after passing through an LKB Uvicord absorptiometer which recorded the transmission at 253 mμm.

Nitrogen was determined by a semi-micro Kjeldahl procedure, phosphorus by the method of Sumner (1944) and sialic acid, calculated as N-acetyl neuraminic acid, according to Warren (1959). The protein content was determined by drying to constant weight at 103 °C a suitable sample of a solution which had been dialysed against a large excess of water.

The effect of rennin on the protein fractions was studied at pH 6·8 and 35 °C using a crystalline preparation of the enzyme. The amount of sialic acid soluble in 12% trichloracetic acid (TCA) at various stages of the reaction was determined as previously described (Beeby, 1963).

Amperometric titration with phenylmercury acetate in 8 M urea at pH 9 (borate), after adding sulphite according to Allison & Cecil (1958), was used to obtain the amount of cystine and cysteine in the samples.

Preparation of the sialic acid-rich fraction

After processing 1500 ml of milk, as described above, approximately 600 ml of crude κ-casein in 0·25 M calcium chloride was obtained. The calcium chloride was removed by gel filtration (Porath & Flodin, 1959). Approximately 150 ml of solution was applied to a Sephadex column (8 × 21 cm), eluted with 0·005 M sodium chloride and the protein collected in approximately 300 ml. To the calcium-poor protein solution (1200 ml) were then added 5 g of ethylenediamine tetraacetic acid (EDTA), the pH adjusted to 7 and the solution held 16 h at 6 °C. The solution was then concentrated under vacuum at 25 °C to a volume of 300 ml and the EDTA removed by gel filtration as described earlier.

Table 1. *The yield of sialic acid in crude κ-casein and in the supernatant obtained by precipitating crude κ-casein with 0·4 M-NaCl at pH 3*

Experiment	Sialic acid in whole casein, %	Sialic acid in κ-casein, %	Sialic acid in κ-casein, % of total	Sialic acid in supernatant protein (N × 7), %	Sialic acid in supernatant, % of κ-casein sialic acid
1	0·40	1·52	75	2·6	22
2	0·53	2·15	53	3·8	52
3	0·46	2·05	66	3·8	27
4	0·44	1·84	74	4·0	80
5	0·46	1·73	62	2·9	45
6	—	1·47	—	2·0	22
7	0·45	2·20	70	4·6	36
8	—	1·80	—	3·8	60
9	0·42	1·94	45	2·5	22
10	0·51	2·06	45	4·4	52
11	—	1·71	—	2·6	80
12	0·44	1·93	55	3·1	44

The calcium-free protein solution was then made 0·4 M with respect to sodium chloride and held 3–4 h (or overnight if convenient) at 6 °C. The solution was then adjusted to pH 3 with N-HCl, warmed to 25 °C and held for 30 min. The precipitate which formed was removed by centrifuging in a Servall RC 2 centrifuge (GSA rotor)

for 20 min. at 7000 rev/min and the supernatant collected. The distribution of sialic acid at the various stages in the procedure is summarized in Table 1.

The sialic acid content of the crude κ-casein varied from 1·47 to 2·20% while 22–80% of this was recovered in the supernatant at pH 3. Redispersing the precipitate and treating again at pH 3 released only small amounts of sialic acid-containing material.

Chromatographic purification of the sialic acid-rich component

The supernatant from the precipitation of κ-casein at pH 3 in the presence of 0·4 M sodium chloride was adjusted to pH 6·5, concentrated under low pressure at 25 °C to a volume of approximately 80 ml, and the salt replaced with 0·1 M sodium acetate at pH 6·5 by running the solution through a Sephadex column (5 × 23 cm) previously equilibrated with the acetate. The protein eluted in some 140 ml which was diluted to 400 ml with 0·1 M acetate and held 16 h at 6 °C.

A DEAE-cellulose column (4 × 12 cm) was prepared by mixing 20 g of the ion-exchanger with 500 ml of 0·1 N sodium hydroxide, allowing to settle and decanting the supernatant. The slurry was packed into a glass column and washed with 500 ml each of water, 0·1 M acetic acid and 0·1 M sodium acetate (pH 6·5) in that order. The column was then cooled to 0 °C and maintained at that temperature during the chromatographic separation.

The cold protein solution was run on to the column. Protein appeared in the effluent after a volume corresponding to the bed volume of the column (140 ml) had run through. When all the protein solution had been run in the column was eluted with 300 ml of the acetate buffer at a flow rate of roughly 5 ml/min. The effluent contained no protein at this stage. The protein not bound to the DEAE-cellulose at pH 6·5 was isolated by concentrating the solution at 25 °C, dialysing exhaustively against water and freeze-drying. This fraction contained approximately 15% nitrogen, 0·11% phosphorus, 0·6% sialic acid and no detectable cystine or cysteine.

The sialic acid-rich fraction was eluted from the column in a linear salt gradient formed from 225 ml of 0·1 M sodium acetate (pH 6·5) and 225 ml of 0·1 M sodium acetate, 0·5 M sodium chloride (pH 6·5). The protein appeared at an effluent volume of around 140 ml and was obtained in a volume of some 200 ml. The solution was adjusted to pH 6·5, concentrated. and after replacement of the salt by 0·1 M acetate, rechromatographed as before. On rechromatographing the sialic acid-rich material eluted at the same place in the gradient and its sialic acid to nitrogen ratio increased to a constant value of around 0·47, usually after 2 runs (Table 2). The solution was then concentrated, desalted by gel filtration and freeze-dried. The protein thus obtained contained 13·0–13·2% nitrogen, 0·96–1·04% phosphorus and 5·9–6·3% sialic acid. Cysteine and cystine were not detected. The yield of protein was 500–600 mg.

When the treatment with EDTA was omitted in the isolation of the κ-casein the supernatant obtained at pH 3 contained disulphide which repeated chromatography failed to remove. The final product in this case contained 5% sialic acid and one disulphide group per 100000 g.

Paper electrophoresis in 0·02 M phosphate buffer at pH 6·7 (16 h at 100 V) revealed that the sialic acid-rich component was negatively charged at this pH. This was to

be expected from the high sialic acid and phosphate content. The protein washed off the paper readily when the conventional staining techniques were employed. It could be located by dipping in an acetone solution of ninhydrin with which it gave a very strong colour. The protein band exhibited considerable spreading.

The protein not bound by DEAE-cellulose was positively charged at pH 6·7.

The results shown in Tables 1 and 2 were obtained during the period March–September 1963. Since that time a greater variation in the sialic acid contents of the chromatographed preparations has been observed. Repeated chromatography of some samples failed to increase the sialic acid to nitrogen ratio above approximately 0·3, which corresponds to around 4 % sialic acid. The crude κ-caseins from which these samples were prepared contained less sialic acid than the corresponding κ-caseins in the earlier experiments.

Table 2. *The effect of chromatography on DEAE-cellulose on the sialic-acid :*
nitrogen ratio of the sialic acid-rich fraction

	Sialic acid: nitrogen ratio after n chromatographic runs			
Experiment	$n = 0$	$n = 1$	$n = 2$	$n = 3$
1	0·280	0·440	0·468	0·470
2	0·184	0·380	0·470	0·471
3	0·226	0·400	0·465	0·465
4	0·325	0·473	0·471	—
5	0·203	0·352	0·455	0·467

The effect of rennin on the sialic acid-rich fraction

The sialic acid-rich protein dissolved readily in water at pH 6·5 to give a clear solution which remained clear at pH 4·7 but which gave a precipitate with 12 % TCA. The proportion of the total nitrogen in the filtrate varied with the concentration of TCA. In a typical case 100, 40 and 25 % of the total nitrogen of a 0·16 % protein solution was found in the filtrates obtained with 4, 12 and 20 % TCA, respectively.

Rennin had no visible effect on the protein and the reaction with the enzyme was followed by determining the proportion of the total sialic acid that was soluble in 12 % TCA as the reaction proceeded. To facilitate precipitation the samples were mixed with casein which had previously been treated with rennin, precipitated at pH 4·7, washed, redissolved and heated at 80 °C for 2–3 min to inactivate any residual enzyme. The final solutions contained approximately 1 % of rennin-treated (para-) casein and 0·1–0·2 % of the sialic acid-rich fraction. The concentration of enzyme used was 4 μg/ml. The controls were the corresponding solutions of para-casein.

The effect of rennin on 10 different preparations was determined in this way. For reasons of clarity the results of only 4 experiments are given in Fig. 1.

Rennin caused an increase in TCA-soluble sialic acid in all samples and after 30 min reaction 80–100 % of the total sialic acid was found in the TCA-filtrate. The proportion of the total sialic acid soluble in 12 % TCA before rennin was added varied from 20 to 70 %. In all cases the ratio of sialic acid to nitrogen in the TCA-filtrate was greater than that of the original sialic acid-rich fraction; in some cases almost double. This ratio was always greater after 30 min reaction than at the start.
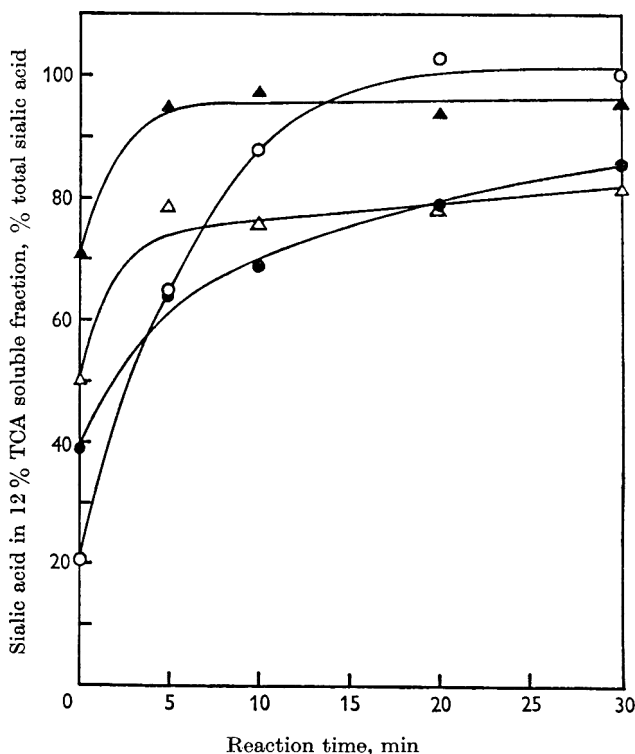
Fig. 1. ○, 0·1 % protein. SA/N ratios: original sample, 0·287; 12 % TCA soluble at 0 min, 0·395; 12 % TCA soluble at 30 min, 0·575. ●, 0·2 % protein, 2 μg/ml rennin. SA/N ratios: original sample, 0·289; 12 % TCA soluble at 0 min, 0·433; 12 % TCA soluble at 30 min, 0·572. △, 0·09 % protein, SA/N ratios: original sample, 0·427; 12 % TCA soluble at 0 min, 0·520; 12 % TCA soluble at 30 min, 0·627. ▲, 0·1 % protein. SA/N ratios: original sample, 0·385; 12 % TCA soluble at 0 min, 0·732; 12 % TCA soluble at 30 min, 0·798.

DISCUSSION

Waugh *et al.* (1960) reported that κ-casein contained cystine, while Beeby (1964) showed that cysteine and not cystine was present in fresh κ-casein preparations. The lack of cystine or cysteine and the recovery of up to 80 % of the sialic acid of κ-casein in the supernatant after precipitating the protein at pH 3 thus further supports the postulate of Beeby & Nitschmann (1963) that κ-casein is a complex.

Since all the sialic acid of κ-casein appears in the material split off by rennin (Beeby, 1963) it follows that the sialic acid-rich fraction isolated from the above supernatant must be, or contain, the component attacked by the enzyme. The release of sialic acid soluble in 12 % TCA from this fraction by rennin supports this supposition. The term $κ_1$-casein will be used for the sialic acid-rich fraction while the material associated with it in the supernatant at pH 3 will be called $κ_2$-casein.

The sialic acid to nitrogen ratios of the chromatographically purified $κ_1$-caseins shown in Table 2 agree well with the value of 0·45 found for the material which is soluble at pH 4·7 after rennin acts on κ-casein and which is also lacking in cystine or cysteine (Beeby, 1963). This similarity in composition suggests that $κ_1$-casein is the material freed from the κ-casein complex by the enzyme. Approximately 50 % of

this soluble material split off by rennin consists of a glycopeptide which is soluble in 12% TCA and which has a molecular weight of the order of 8000 (Nitschmann, Wissman & Henzi, 1957; Jollès & Alais, 1960; Jollès, Alais & Jollès, 1961). If κ_1-casein is a single protein its molecular weight would therefore be around 16000, which agrees with the postulate of Beeby (1963) that κ-casein consists of 3 units each with a molecular weight of about 16000 and that only one of these units contains sialic acid.

As all the sialic acid of κ-casein is found in the glycopeptide (Beeby, 1963) this would be expected to have a sialic acid content double that of κ_1-casein or 8–12·6%. These values are a little lower than the values of 11·3–14·3% reported for the glyco-peptide by other workers (Nitschmann et al. 1957; Brunner & Thompson, 1959; Jollès et al. 1961). It is not clear whether the differences in the sialic acid contents of the κ_1-casein preparations arise from contamination or are due to natural variation, either genetic, environmental or seasonal.

The reason for the rather wide variation in the proportion of the κ-casein sialic acid recovered in the supernatant at pH 3 (Table 1) is not apparent. However, it most probably lies in differences in the state of aggregation of the protein. This is supported by the observation that κ-casein contains sulphydryl groups which are masked. Conditions similar to those employed in freeing κ_1-casein temporarily unmask the SH-groups although these become unavailable again not through oxida-tion but presumably because they are once more buried within a tightly formed complex (Beeby, 1964).

The action of rennin on κ_1-casein is generally similar to the attack on κ-casein by the enzyme in that most of the sialic acid appears in the 12% TCA-soluble fraction after some 30 min and the ratio of sialic acid to nitrogen in the TCA-filtrate increases as the reaction proceeds. However, within the reaction times studied the sialic acid to nitrogen ratio of the TCA-filtrate did not reach a value sufficiently high to be able to say with reasonable certainty that the glycopeptide (sialic acid:nitrogen 0·9–1·0) is formed from κ_1-casein. Because the ratio in the TCA-filtrate at zero time was always higher than the corresponding κ_1-casein (Fig. 1) it is clear that either the protein is heterogeneous or the TCA ruptures some labile bond or bonds in it.

Although the work presented in this paper poses many questions which must be answered before definite conclusions may be drawn regarding the role of these 2 new fractions of κ-casein, their isolation should facilitate a more complete understanding of this important complex, and ultimately of the chemical structure of the casein micelle.

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A method for studying the factors in milk which influence the deposition of milk solids on a heated surface

By H. BURTON

National Institute for Research in Dairying, Shinfield, Reading

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SUMMARY. A laboratory method is described which it is hoped will be of value in the search for some of the chemical factors in milk which control the formation of deposits on heated surfaces during commercial processing. The method uses an electrically heated platinum wire as the surface on which deposits are formed under controlled heat transfer conditions. Preliminary results are given on the effect of milk pH on deposit formation, differences in the sensitivity to deposit formation of different bulk milk supplies, and the effect of forewarming on the reduction of deposition.

The importance of the problem of deposit formation from heated milk has been discussed briefly in a previous paper (Burton, 1961). It is known that the rate of deposition of milk solids in a heat-treatment plant depends in practice on such factors as the milk velocity, and the temperature difference between the heating medium and the milk (Gynning, Thomé & Samuelsson, 1958). These factors operate by influencing the thickness of and the temperatures in the stationary milk layer at the heating surface.

Two other factors are likely to be important. First there is the chemical composition of the milk: it is known, for example, that the acidity of the milk has a considerable effect on the amount of deposit formed (Gynning *et al.* 1958; Burton, 1961). Secondly there is the nature of the interaction between the deposit and the surface on which it forms, which is likely to determine the ease of formation of the first deposit layer. Pflug, Hedrick, Kaufmann, Keppeler & Pheil (1961) have shown that the nature of the surface on which the deposit is formed has an effect on the rate of formation of a film of milk solids from pasteurized milk. Jennings (1961) has reviewed the evidence to suggest that there is an adsorption phenomenon involved at the surface on which the first stages of deposition occur, and that the deposition conditions and cleaning requirements may be modified by saturating possible adsorptive sites with non-milk material.

The laboratory method for investigating the rate of deposit formation described previously (Burton, 1961) was suitable for determining the effect of the surface on which the deposit was formed, although as described the method used only a stainless steel surface. However, the method was very sensitive to variations in the amount of dissolved air in the milk. This made it unsuitable for comparing samples which

were likely to contain different amounts of dissolved air. In particular, it made it impossible to determine the effect of forewarming processes which have been used in practice to reduce the amount of deposition from milk during heat treatment (Bell & Sanders, 1944), since these often involve an incidental loss of air.

A new method has therefore been developed in which the rate of formation of deposits from milk on a heated surface can be studied under controlled heat transfer conditions. The effect of various chemical and physical modifications to the milk on the rate of deposition can be studied with relatively small quantities of milk (350 ml), under temperature conditions which are similar to those through which milk normally has to pass during high temperature heat treatment. It is hoped in this way to determine the factors inherent in the milk which have an influence on the deposition problem in commercial processing equipment. As will be seen, the new method depends on the heating surface being platinum, so that it is unable to give any information on the effect of different surface materials on the deposition phenomena.

McAdams, Addoms, Rinaldo & Day (1948) introduced a method for determining the heat transfer conditions from heated horizontal wires to boiling water. This involves the use of a platinum wire through which an electric current is passed. If the wire is immersed in water, the heat developed by the electric current flow is transferred to the water, and the wire temperature becomes higher than the water temperature by the amount necessary to give the corresponding transfer of heat. The platinum wire can be calibrated so that its temperature can be obtained from its resistance. The difference in temperature between the wire and the water in which it is immersed can then be found. The corresponding rate of heat transfer can be found from the power input to the wire.

Van Stralen used this method to study the rate of heat transfer to boiling binary liquid mixtures (1959) and also to boiling separated milk (1956). The latter paper includes data on the increase in the temperature of the heating wire at a constant rate of heat transfer to boiling milk, due to the build-up of deposits on the wire which progressively reduce the heat transfer coefficient. These conditions, in which the deposit formation is aggravated by nucleate boiling at the heated surface, do not represent the practical situation within a heat exchanger, where boiling is normally prevented by the application of back pressure to the milk through restriction of the outlet. However, the method may be used with suitable modifications to follow the formation of deposits under practical temperature conditions and with boiling prevented by the application of pressure. This paper describes the modified method, and gives some of the first results obtained.

APPARATUS AND METHOD

Apparatus

A simplified diagram showing the apparatus used for milk, and the associated measuring circuit, is shown in Fig. 1. The milk vessel (1) (6 in. high × 3 in. int. diam.) is made from a length of glass pipeline (Q.V.F. Ltd., Stoke-on-Trent). Both the base (2) and lid (3) are machined stainless steel plates and each is held in position by a backing flange with a rubber gasket. The lid supports 2 stainless steel pillars (4), 4 in. long and $\frac{3}{8}$ in. diam., insulated from the lid by rubber grommets and projecting down into the vessel at $2\frac{3}{8}$ in. centres. The lower ends of the pillars form screwed terminal

blocks in which the ends of the platinum wire are clamped between flat surfaces. The platinum wire (5) is 36 s.w.g. pure platinum (0·0076 in. diam.), and has a total length of 70 mm. The wire is clamped into the terminal blocks so that the ends of the wire are flush with the outside edges of the pillars, being clamped across the whole width. The effective heating length is therefore about 50 mm, and the effective wire resistance is approximately 0·26 Ω at 65 °C. The upper ends of the pillars, above the lid, are screwed and have stainless steel nuts, to take the electrical connections.
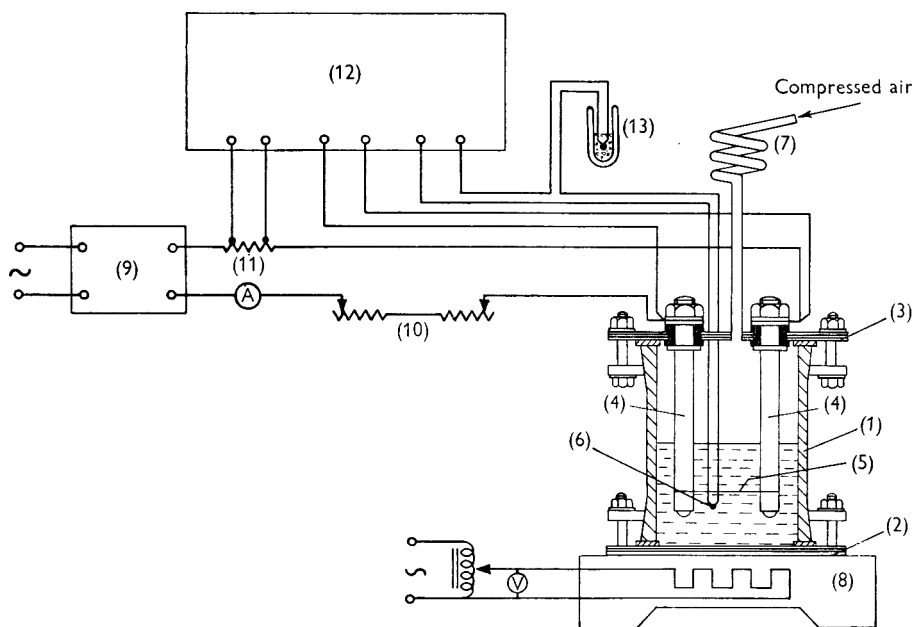
Fig. 1. Diagram of experimental apparatus: (1) milk vessel, (2) machined base, (3) machined lid, (4) stainless steel pillars, (5) platinum wire, (6) thermojunction, (7) condenser coil, (8) hot plate, (9) stabilized power unit, (10) current control resistors, (11) standard resistor, (12) vernier potentiometer, (13) cold junction.

A copper-constantan thermocouple, sheathed in stainless steel, passes through the lid in a bushing, and is placed so that the thermojunction (6) is about 1 in. below the centre of the platinum wire.

Brazed into the lid is the lower end of a stainless steel coil (7) which acts as a simple air-cooled reflux condenser. Compressed air is supplied to the inside of the vessel through the coil, to prevent boiling of the milk at the heating wire surface.

The lid also has a screwed opening, not shown in the diagram, through which the vessel can be filled or emptied. This is normally closed with a knurled plug.

The vessel and its contents are maintained at a constant temperature with a hot plate (8), supplied through a variable auto-transformer by which its temperature can be controlled. To limit heat losses, the vessel is surrounded by a stainless steel shield, not shown in the diagram.

The wire heating current is supplied from a stabilized power unit (9), capable of supplying 5A d.c. at up to 15 V. The value of the current is controlled by the output of the power pack and the coarse and fine variable resistors (10). A 0·1 Ω standard

resistor (11) is included in the heating wire circuit for accurate measurement of the current flowing; the reading of an ammeter is used as the basis for control of the current to a constant value. The heating wire circuit is isolated from earth to minimize leakage current flow between the wire and its supports and the base of the vessel, which otherwise would cause local pH changes and hence coagulation of the milk protein.

The potential differences across the platinum heating wire and the standard resistor are measured by a vernier potentiometer (12), to an accuracy of about 2×10^{-4} V for the resistor and 5×10^{-1} V for the wire. The potential across the standard resistor is used to calculate the heating current, by Ohm's Law. The wire potential divided by the wire current gives the wire resistance, which can be converted to wire temperature by the calibration procedure and calculations described below.

The thermocouple is connected to a cold junction (13), cooled by ice and water in a vacuum flask, and the differential e.m.f. is measured with the vernier potentiometer (12) to 1 μV. The temperature of the liquid in the vessel can be derived from the thermocouple calibration.

Three hundred and fifty ml of milk is used for each experiment, so that the heating wire is then approximately 1 in. below the milk surface. Although milk is an ionic conductor, its resistance in parallel with the platinum wire is sufficiently high for there to be no shunting effect.

A new piece of platinum wire is used for each test. Each piece of wire is annealed before use, at orange heat for approximately 10 min, by passing a low voltage alternating current through it.

Calibration of the thermocouple

Before installation, the thermocouple was calibrated in an oil bath whose temperature was controlled by a contact thermometer and relay, with an NPL certificated thermometer as the standard. Measurements were made at various steady temperatures between 62 and 103 °C. Within this range, the variation of e.m.f. with temperature was linear, the relationship being expressed by

$$\text{Temperature } (°C) = (x + 375 \cdot 3)/46 \cdot 297, \tag{1}$$

where x is the potentiometer reading in μV.

Calibration of the platinum heating wire

Three hundred and fifty ml of distilled water was placed in the heating vessel, and was heated by the hot plate to 93 °C. At a series of wire currents between 0·03 and 0·10 A, low enough for the wire temperature to be little different from that of the surrounding water, the wire resistance was determined from the potentials across the wire and the standard resistor. It was found that there was a slight drop in resistance between 0·03 and 0·05 A, but that the resistance remained constant between 0·05 and 0·10 A. It followed that for any current between 0·05 and 0·10 A the wire temperature could be considered to be the same as that of the water.

With the same piece of wire and a constant current of 0·10 A as read on the ammeter, the wire resistance was determined for a series of different water temperatures between 72 and 102 °C. A linear variation of resistance with temperature was found over this range.

Assuming a first order variation of resistance with temperature over the temperature range of interest in these experiments, we have

$$R_\theta = R_\Theta \left[1 + \alpha \left(\theta - \Theta \right) \right], \tag{2}$$

where R_Θ is the resistance at some reference temperature Θ, R_θ is the resistance at a higher experimental temperature θ, and α is the temperature coefficient of resistance. For a linear variation of resistance with temperature as found, the effective value of α varies with temperature. For 10 °C ranges of temperature, the temperature coefficients of resistance calculated from the resistance-temperature data were:

$$\alpha_{80-90\,°C} = 0\cdot00302,$$
$$\alpha_{90-100\,°C} = 0\cdot00292,$$
$$\alpha_{100-110\,°C} = 0\cdot00283.$$

For the calculation of the experimental results, equation (2) is more conveniently written:

$$\theta - \Theta = (R_\theta / R_\Theta - 1)/\alpha. \tag{3}$$

Experimental method and calculations

To preheat the apparatus and to enable the resistance of each experimental platinum wire to be determined at some base temperature, the test vessel is filled with 350 ml distilled water and brought to a suitable temperature by means of the hot plate. The preheating temperature should be similar to the milk temperature to be used. Time can be saved by filling the vessel with water which is already at a suitable temperature, so that time for equilibration rather than heating is all that is needed.

A low current of about 0·1 A is passed through the wire, and the voltages across the standard resistor and the wire are measured. The resistance of the wire is derived from those voltages and the value of the standard resistor. The corresponding temperature readings are the basic values required for subsequent calculation.

For a milk temperature during the experiment of 63–64 °C, 350 ml of milk is preheated to about 65 °C in an external vessel and then transferred into the milk vessel. The knurled stopper to the access hole is replaced, and the compressed air supply is turned on. It is most important that this should be done before the heating current is applied, as otherwise boiling may take place on the wire and a very rapid and abnormal deposition will occur—an air pressure of 25 lb/in.² has been found suitable. The heating current is then switched on, and the timing of the experiment started.

A heating current of 4·5 A with the wire resistance of about 0·26 Ω gives a heat flux from wire to milk of about 4·4 cal sec⁻¹ cm⁻², and results in a wire-milk differential temperature of 28–29 °C for a clean wire. For a milk temperature of 63–64 °C, the wire temperature will therefore be about 92 °C at the start of a test, and will steadily rise as milk deposits form and insulate the wire thermally. The wire current is maintained as nearly as possible constant throughout the experiment by adjustment of the variable resistors if necessary. The wire resistance rises throughout an experiment as the wire temperature rises, but only by some 1 %, so the increase in the heat input to the system is negligible.

The amount of deposit formed on the wire during an experiment is small. For example, 68 wires from the same number of experiments, each with standard heat transfer conditions and lasting for 90 min, were found to have an average of 0·63 mg dried deposit on each wire.

The milk and wire temperatures are to some extent chosen arbitrarily, and can be varied if necessary to suit different experimental requirements. As will be shown later, forewarming of the milk sample reduces the amount of deposition: the milk temperature during the test should therefore not be so high that forewarming during the experiment interferes with the results. At the same time, the milk temperature should not be so low that a practicable energy input to the wire cannot bring the wire temperature up to a value to cause deposit formation. The conditions given here are a reasonable compromise, but they are not necessarily ideal for all experimental purposes.

At suitable time intervals during the experiment measurements are made of the thermocouple voltage, the voltage across the standard resistor, and the voltage across the heating wire. The calculation of the wire-milk differential temperature at each time is made as follows.

Assume that the basic temperature and wire resistance, obtained in the preheated water, are Θ and R_Θ, respectively. If the wire resistance given by the experimental observations is R_θ, corresponding to a wire temperature θ, then the temperature difference from equation (3) is

$$\theta - \Theta = (R_0/R_\Theta - 1)/\alpha.$$

The value of α to be used in this calculation depends on the basic and final wire temperatures. For the operating conditions given here, $\alpha = 0·00302$ is a suitable value. From the basic temperature Θ, the true wire temperature can be found. The milk temperature θ_m is obtained from the thermocouple e.m.f. by applying equation (1), and the wire-milk differential temperature

$$\Delta\theta = \theta - \theta_m$$

is then found by subtraction.

The results may be plotted in the form of a graph of $\Delta\theta$ against t, the time in minutes from the beginning of the experiment. It is shown in the Appendix that, with simplifying assumptions, $\Delta\theta$ should rise linearly with t at a slope determined by the rate of deposition of milk solids on the wire. However, in practice, a linear variation is rarely obtained, except when the rate of deposition is very low, and the curves are concave downwards. A plot of $\Delta\theta$ against $\sqrt{t}$ is, however, linear and its slope K may be taken as a measure of the rate of deposition. The reason why the curves should become linear when $\sqrt{t}$ is used as the abscissa is obscure. It is unlikely to be related to the cylindrical geometry of the experimental system, since the thickness of the deposit layer formed is not large in relation to the wire diameter. A typical deposit layer is only about 0·002 in. thick on a wire of 0·0076 in. diam., so that the plane geometry considered in the Appendix should give a close approximation to the practical case. The use of the $\sqrt{t}$ plot is therefore essentially empirical to enable better comparison between experiments to be obtained.

PRELIMINARY INVESTIGATIONS

Heat transfer to distilled water

To test the experimental apparatus, a series of experiments was made on heat transfer rates from the platinum wire to distilled water. Three hundred and fifty ml of distilled water was equilibrated in the heating vessel at approximately 100 °C. The basic platinum wire resistance was determined by passing a small current through the wire as described above. The wire-water differential temperature was then determined at a series of wire currents from 1·4 to 4·5 A. For each current, the heat flux in cal sec^{-1} cm^{-2} was calculated from the current, wire resistance and wire surface area.
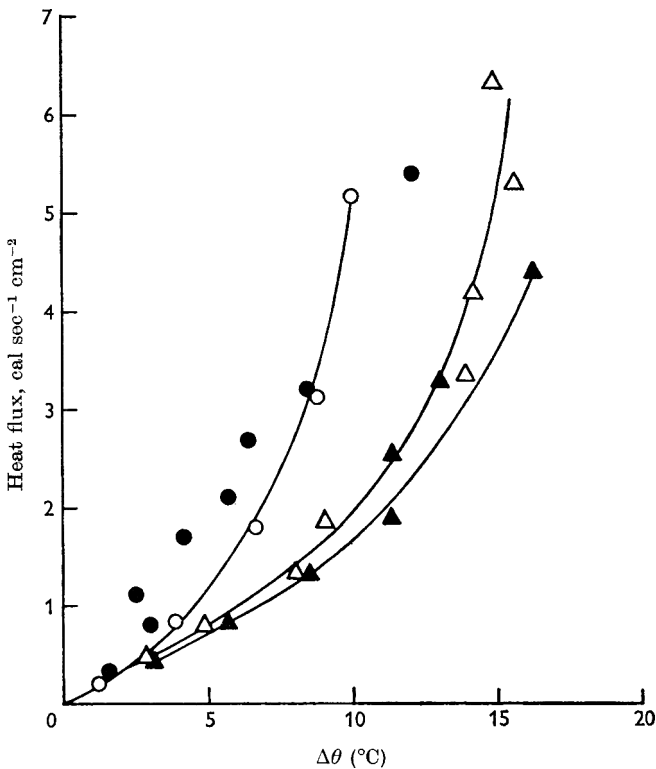
Fig. 2. Variation of heat flux with differential temperature between electrically heated Pt wire and distilled water at about 100 °C. ○, Water temperature 95–96 °C, zero back pressure; ●, zero back pressure (van Stralen, 1956); △, water temperature 99–102 °C, 10 lb/in² back pressure; ▲, water temperature 96–102 °C, 15 lb/in² back pressure.

Each series of measurements was taken at 0, 10 and 15 lb/in.² back pressure. The variation of heat flux with differential temperature is shown in Fig. 2. The results obtained at zero back pressure are very similar to those given for similar conditions by van Stralen (1956), and included for comparison in Fig. 2.

The importance of back pressure in relation to wire temperature

In some experiments the milk temperature was held between 85 and 90 °C, while the air pressure was controlled at 25 lb/in.². Under these conditions, the wire tempera-

ture could approach the temperature of boiling milk at 25 lb/in.². A $\Delta\theta - t$ curve of the form shown in Fig. 3 was then obtained. The slope was uniform over the early stages of the experiment, but became steeper very rapidly as boiling conditions at the wire surface were approached. Boiling at the wire surface must therefore be avoided by suitable choice of operating conditions if comparable results are to be obtained in different experiments.

A curve showing the variation of wire temperature with time is also shown in Fig. 3. It can be seen that the curve begins to turn rapidly upwards at a wire temperature about 10 °C below the boiling point.
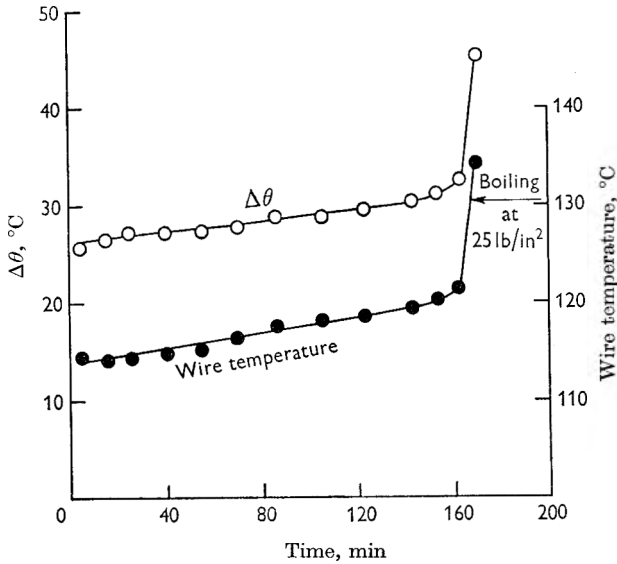
Fig. 3. Variation of wire temperature and wire-milk differential temperature with time, as boiling is approached. Milk temperature 87–89 °C, wire current 4·5 A, back pressure 25 lb/in.².

In a series of experiments of this kind, the deposit curve gave a sharp upward turn at wire temperatures from 6 to 12 °C below the boiling point. It is probable that at these threshold temperatures, steam or air bubbles separate from the milk at the wire surface, and rapid deposition occurs on these nuclei, perhaps partly due to surface denaturation.

It follows that, in practical heat exchangers operating with milk temperatures near to or above atmospheric boiling point, a back pressure must be applied which corresponds to a temperature higher than the maximum milk temperature if heavy deposition is to be avoided. Over the practical range of temperatures likely to be used, an excess back pressure of 15–20 lb/in.² will raise the boiling point by 15–20 °C. This will ensure that the maximum milk temperature is not within that 6–12 °C range below the boiling point where exceptional deposition begins and will also ensure that dissolved air remains in solution (Burton, 1958).

Reproducibility of results

Pairs of deposit curves for different milks, tested a second time within not more than 4 h of the first test, are shown in Fig. 4. Milks (*a*) and (*b*) were fresh separated milks

from the Institute's farm. Milk (c) was a commercial bulk milk sampled cold at a dairy and separated cold at the Institute before test.

The linear shape of the curve obtained when $\sqrt{t}$ is used as the abscissa is clearly shown.

The extrapolated values of $\Delta\theta$ at zero time vary. The differences in the extrapolated values in Figs. 4 (a) and (b) have been shown by experience to be typical of those found in repeated experiments, but the difference in Fig. 4 (c) is high. It is believed that the differences are caused by slight variations in the shape of the platinum wire between the mounting pillars, and kinks in the wire must be avoided. The differences in the extrapolated values of $\Delta\theta$ at zero time have a negligible effect on the K values of the curves, on which comparisons have been based.
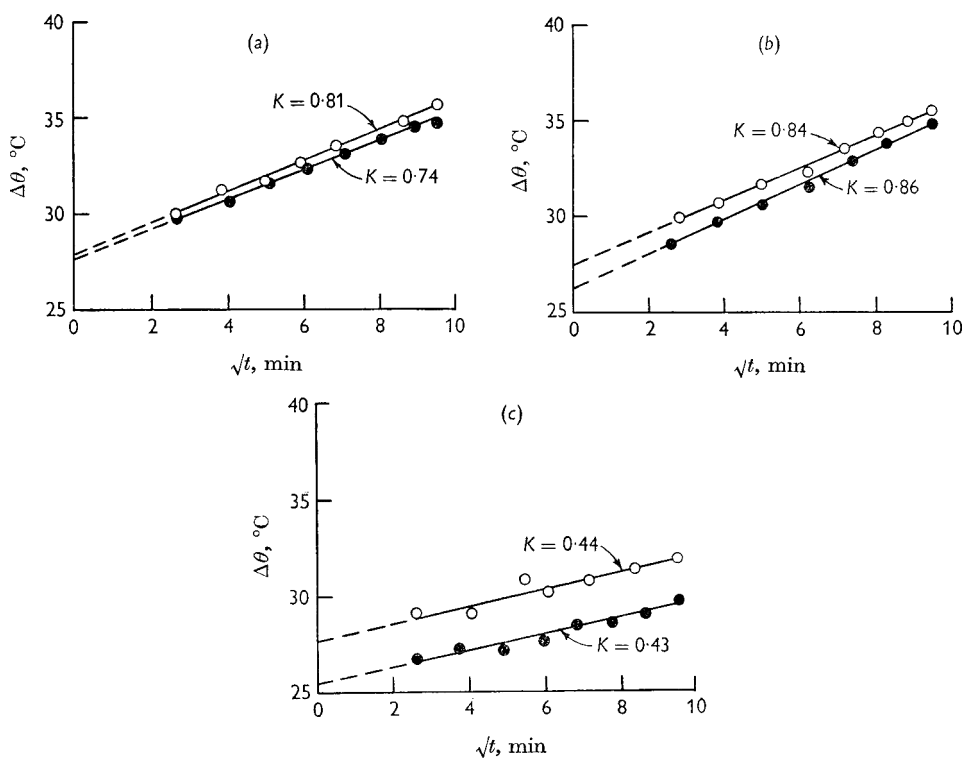
Fig. 4. Reproducibility of deposit curves (K = slope) in repeat tests with the same milks: (a) and (b), milks from Institute farm; (c) commercial bulked milk. Milk temperature, 63–65 °C; wire current, 4·5 A; back pressure, 25 lb/in.²

THE EFFECT OF THE pH OF MILK ON THE RATE OF DEPOSIT FORMATION

Bulk milk was taken from the Institute's farm, stored at about 5 °C for 24 h and then separated cold. Three hundred and fifty-ml quantities of separated milk were adjusted to pH values of 6·40, 6·50 and 6·62 by the addition of dilute HCl. The unadjusted milk had a pH value of 6·76.

Each sample was tested using the method and standard operating conditions given above. The deposit curves obtained are shown in Fig. 5. The variation of the corresponding K values with pH is shown in Fig. 6.

The sensitivity to deposit formation varies slightly but definitely with pH over the range of pH values likely to be found with fresh milk. However, if the pH falls, e.g. because of bacterial action, the sensitivity to deposit formation increases rapidly.
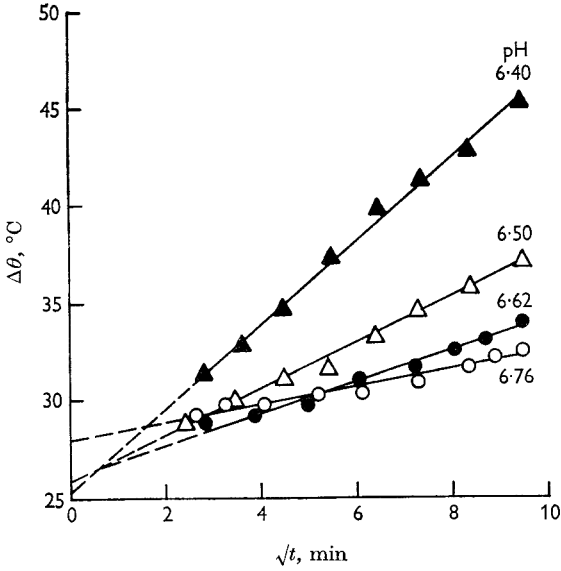
Fig. 5
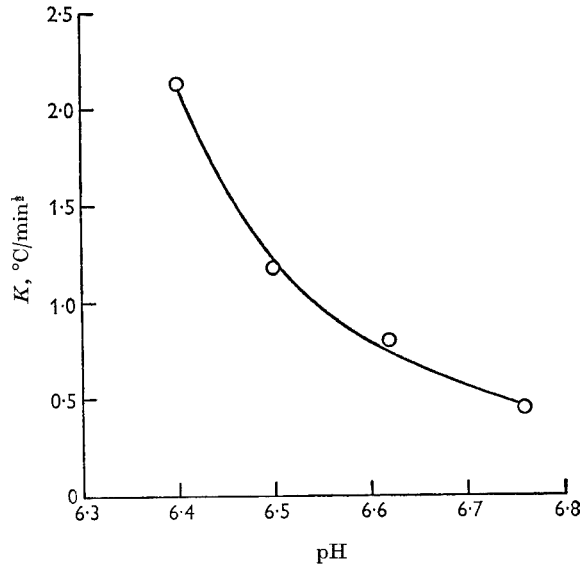
Fig. 6

Fig. 5. Effect of pH on deposit curves.

Fig. 6. Variation of deposit formation with pH, for the same milk with pH adjusted by addition of HCl.
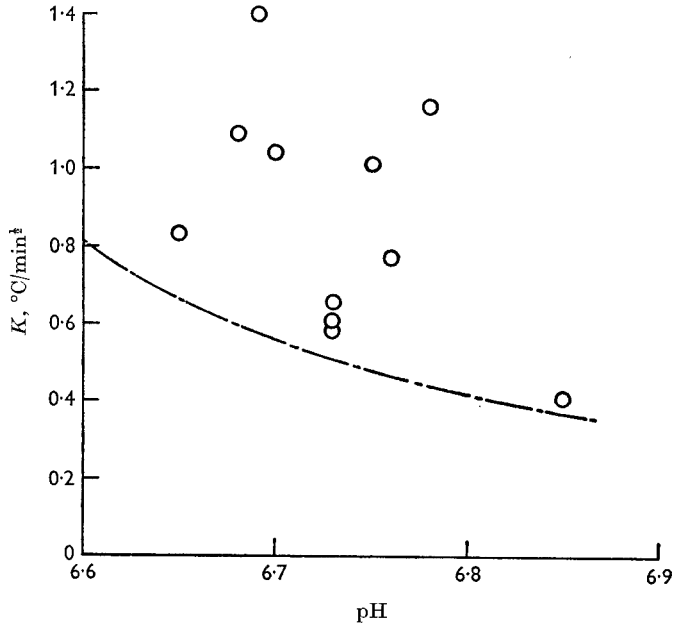
Fig. 7. Variation of deposit formation with pH for commercial bulk raw milks.
— · —, Curve from Fig. 6.

To determine the natural variations between different milk supplies, milk was sampled cold from the storage tanks at a large dairy over a period of 4 months. The milks were transported cold to the Institute, separated cold, and tested on the day of sampling by the standard technique. The pH of each sample was measured with a glass electrode. The results are shown in Fig. 7, together with a section of the pH curve given in Fig. 6, extrapolated to pH 6·85.
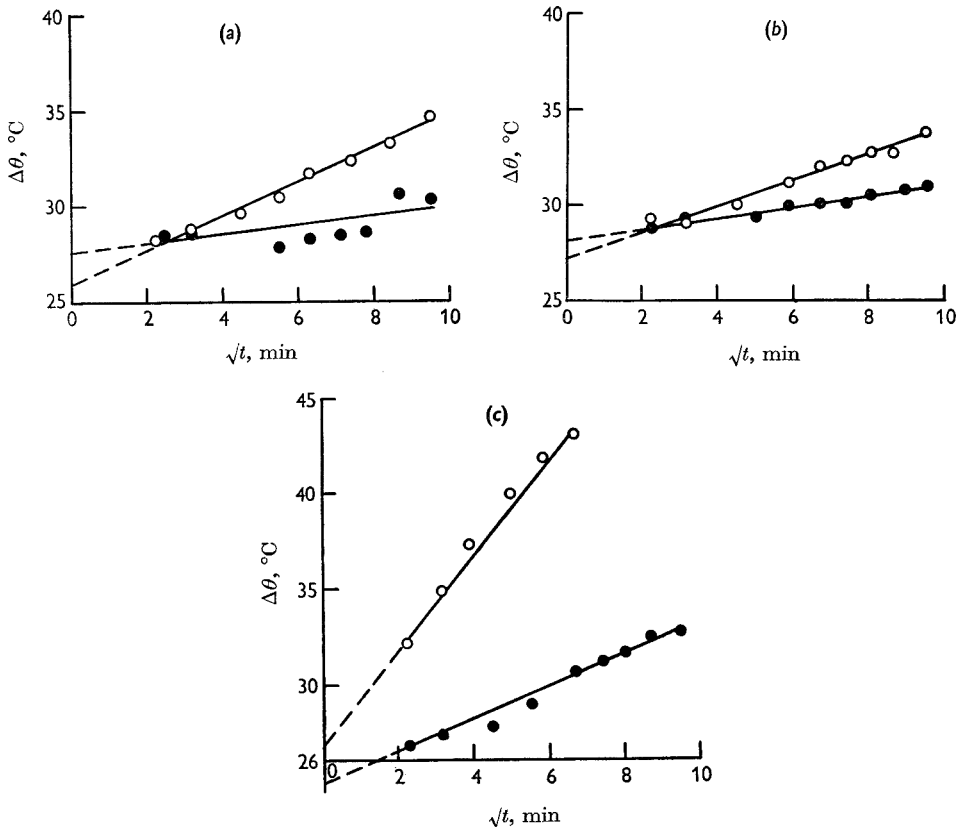
Fig. 8. Effect of forewarming of milk on deposit formation (a) ○, control, 24-h old; ●, forewarmed, 85 °C for 5 min. (b) ○, control, 48-h old; ●, forewarmed, 80 °C for 5 min. (c) ○, control, 72-h old; ●, forewarmed, 85 °C for 5 min.

There are wide variations in the sensitivities of the bulk milks to deposit formation, which seem to be little related to their pH variations. All the results were higher than were given by the fresh, farm bulk milk for which the curve of Fig. 6 was obtained. It appears that there is some factor in raw bulk milk other than pH which can increase its sensitivity to deposit formation, although the milk is acceptable for dairy intake. Further studies are needed to identify this factor.

THE EFFECT OF FOREWARMING ON RATE OF DEPOSIT FORMATION

When milk is to be treated in indirectly heated plants it has become common practice to forewarm it at a temperature of about 85 °C for 2–6 min before it is heated to 100 °C and above. This forewarming treatment appears to reduce the amount of

deposit formation, but its effect has not apparently been demonstrated in the laboratory.

Bulk herd milk was obtained from the Institute's farm, separated, and stored at about 5 °C for up to 3 days before use. For each experiment, a control sample of 350 ml was preheated to 65 °C and a test immediately started.

For the forewarmed samples, 350 ml of milk was heated with rapid agitation in a stainless steel beaker to either 85 or 80 °C in about 7 min and held at this temperature for 5 min. It was then run into a glass beaker and cooled to below 60 °C in flowing tap water with rapid agitation. The milk was then returned to the stainless steel beaker and heated again to 65 °C, when the test was immediately started.

The results of 3 experiments are shown in Fig. 8.

Forewarming for 5 min at both temperatures gave a marked reduction in the amount of deposition. Comparison of the K values for Figs. 8(a) and (b) suggests that forewarming at 85 °C for 5 min reduced the deposition by about 70%, whilst forewarming at 80 °C for 5 min reduced the amount by about 60%. No deposit was visible in the stainless steel beaker used for the forewarming. It appears that there was a true reduction in the amount of deposit produced, and the missing deposit did not appear elsewhere.

The results in Fig. 8(c) are interesting apart from the demonstration of the effect of forewarming. This milk had been held for 72 h in a refrigerator before test, and its smell suggested that bacterial growth had occurred. The control sample showed a greatly increased amount of deposit compared with that of the same milk when only 24-h old (Fig. 8 (a)). Forewarming reduced the amount of deposit under these extreme conditions also, and it appears that the proportionate reduction was similar to that given by the same forewarming treatment of the 24-h-old milk.

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Theory of the rise in wire temperature caused by deposit formation

Symbols: θ = Temperature of wire.

θ_m = Temperature of milk.

θ_s = Temperature of deposit-milk interface.

l = Thickness of deposit.

k_d = Thermal conductivity of deposit.

k_m = Heat transfer coefficient for deposit-milk interface.

C = Rate of heat input to wire.

D = Constant, representing sensitivity of milk sample to deposit formation.

t = Time.

Assume for simplicity a plane heating surface having unit surface area. Then for the deposit layer the rate of heat transfer is $k_d(\theta - \theta_s)/l$. But this must equal the rate of heat input to the wire so that

$$C = \frac{k_d(\theta - \theta_s)}{l}. \tag{1}$$

For the deposit-milk interface the rate of heat transfer is $k_m(\theta_s - \theta_m)$ which must also equal the rate of heat input so that

$$C = k_m(\theta_s - \theta_m)$$

or

$$\theta_s = \frac{k_m \theta_m + C}{k_m}. \tag{2}$$

Substituting this expression for θ_s in (1) we obtain

$$C = \frac{k_d}{l}\left[\theta - \left(\frac{k_m \theta_m + C}{k_m}\right)\right].$$

Therefore

$$\theta = \frac{Cl}{k_d} + \frac{k_m \theta_m + C}{k_m}. \tag{3}$$

Differentiating with respect to time, since the second term in (3) is constant, gives

$$\frac{d\theta}{dt} = \frac{C}{k_d} \cdot \frac{dl}{dt}. \tag{4}$$

The way in which the deposit thickness varies with time is not known. Assume that the rate of deposition of material is proportional to the temperature at the surface of deposition, so that

$$\frac{dl}{dt} = D\theta_s.$$

Substituting θ_s from (2)

$$\frac{dl}{dt} = \frac{D}{k_m}(k_m \theta_m + C).$$

Substituting for $\dfrac{dl}{dt}$ in (4) we have

$$\frac{d\theta}{dt} = \frac{CD}{k_d k_m}(k_m \theta_m + C).$$

Integrating,

$$\theta = \frac{CD}{k_d k_m}\,(k_m \theta_m + C)t + \ \text{constant}. \tag{5}$$

Now from (3) and (5), when $t = 0$ and $l = 0$,

$$\theta = \frac{k_m \theta_m + C}{k_m},$$

therefore

$$\theta = \frac{CD}{k_d k_m}\,(k_m \theta_m + C)t + \frac{k_m \theta_m + C}{k_m},$$

therefore

$$\theta = \left(\theta_m + \frac{C}{k_m}\right)\left(1 + \frac{CD}{k_d}t\right).$$

If the difference $(\theta - \theta_m)$ is denoted by $\Delta\theta$, at zero time $\Delta\theta_0 = C/k_m$, and so is dependent principally on the rate of heat input to the wire. Subsequently, $\Delta\theta$ will rise linearly with time if the experimental factors θ_m and C are constant and the heat transfer coefficients k_m and k_d are constant. The slope of the $(\Delta\theta - t)$ curve will depend on the sensitivity of the milk to deposit formation, represented by D. Thus the sensitivity can be derived from the shape of the curve.

The relationship in the cow between the osmotic pressure of milk and of blood

By J. V. WHEELOCK, J. A. F. ROOK and F. H. DODD

National Institute for Research in Dairying, Shinfield, Reading

SUMMARY. Similar, marked variations in the freezing-point depressions of jugular-venous blood and of milk throughout a day were observed in cows when drinking-water was offered for a single, short period each day. Values for milk were found to agree more closely, however, with those for mammary-venous blood than with those for jugular-venous blood. It appears that milk is in osmotic equilibrium with the blood flowing through the udder continuously throughout the period the milk remains within the udder and not only during its formation, and that milk secretion causes a slight alteration in the osmotic pressure of fluids within the immediate locality of the mammary gland. Changes in the milk composition that occurred in association with the observed changes in freezing-point depression were consistent with a movement of water into or out of the udder in response to any change in the osmotic pressure of blood.

The osmotic pressure of the milk of cows under normal conditions of feeding and management, as it is reflected by the convenient measurement of freezing-point depression (Δ) in °C, is close to that of blood (Winter, 1896; Hortvet, 1921) and shows a constancy similar to that found for blood and other body fluids (Dittmer, 1961). As early as 1915, however, van der Laan (1915) observed that the Δ of the milk increased by 8 % after a cow was given orally 1 kg of sodium sulphate. Twenty-two hours later the cow was offered water, drank 45 kg and the Δ then decreased to the original value. Aschaffenburg & Rowland (1950) and Hillman, Provan & Steane (1950) reported instances of abnormal morning-evening differences in the Δ and in the chemical composition of the genuine milk of herds of cows. These differences were attributed to the herds having access to water for a limited period only each day and to the animals consequently drinking most of their daily requirement immediately water was available. Aschaffenburg (1955) later demonstrated experimentally that milking cows, offered drinking-water over a single period of 1 h each day, showed marked variations throughout the day in the Δ of their blood and unusually large morning-evening differences in the Δ of their milk.

Restricting the period of access to drinking-water for milking cows offers, therefore, a simple experimental technique for inducing marked variations in the osmotic pressure of blood and milk. In this paper experiments are reported in which the technique has been used to study the relationship between the osmotic pressure of blood and milk and, in particular, whether milk is in osmotic equilibrium with blood only during milk formation, or continuously throughout the period it remains within the udder.

Five of the 6 experiments described had the same basic design. In expts. 1–5, lactating cows accustomed to receive their drinking-water at 24-h intervals were milked at short intervals throughout an experimental period varying in duration from 6–30 h, and in expts. 1–3 serial samples of jugular-venous blood also were taken over the same periods. Freezing-point determinations made on these samples measured the progressive changes in osmotic pressure of the blood during the intervals between water ingestion and the associated changes in the osmotic pressure of milk within the udder. In the 6th experiment both lactating and non-lactating cows with unrestricted access to water were used to measure the Δ's of samples taken simultaneously from blood of the jugular vein, from blood of the mammary vein and, in lactating cows, from milk.

Table 1. *The lactose contents of milk (g/100g) from the separate quarters of the udders of 3 cows used in expts. 1–5, at a milking before the commencement of experiments*

	Quarter			
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Cow	Right fore	Left fore	Right hind	Left hind
49	4·60	4·52	4·58	4·59
66	4·53	4·54	4·53	4·54
020	4·37	4·65	4·61	4·46

Animals and management

Three lactating, Friesian cows (49, 020 and 66) giving 20–25 kg of milk daily were used for expts. 1–5. Cows 49 and 66 were in their 2nd lactation and bacteriological examinations of the secreted milk had not detected any udder infection. Cow 020 was in her 6th lactation and had a record of frequent infections in the right fore and left hind quarters in previous lactations, and in the first routine bacteriological test after the completion of the present experiments infections were again found in these quarters. The lactose contents of the milk from the separate quarters of the udder for the 3 cows at a milking before the commencement of experiments are given in Table 1. The results show a similar composition for all 4 quarters in cows 49 and 66, but the right fore and left hind quarters in cow 020 showed lower lactose contents than the other 2 quarters, the result presumably of previous bacterial infection.

The animals were housed in loose-boxes and given a diet of hay and a concentrate mixture balanced for milk production. The daily allowances of hay and concentrates were divided into equal portions, the number depending on the daily frequency of milking, and a portion given at each milking. Except during experimental periods, the management and milking routines were normal.

For expt. 6, 4 lactating and 2 non-lactating Friesian cows were taken from the Institute herd.

EXPERIMENTAL PROCEDURE

Several days before the start of expts. 1–5 drinking-water was offered at a fixed time each day for a period of 15 min. By the 2nd day of water restriction, animals consumed their usual daily intake of water (50–70 kg) in about 5 min. Short-interval

(3-hourly or 6-hourly) milking, instead of the usual twice-daily milking, was introduced 3 or 4 milkings before the start of an experiment. During experimental periods a milking machine designed for the separate collection of the milk from the 4 quarters of the udder was used and at certain of the milkings (see Table 2) either 1 or 2 quarters were left unmilked. Whenever a quarter was milked a sample was taken. In expts. 4 and 5, at the 1st milking after the ingestion of water the milk from 2 quarters, one

Table 2. *Details and sequences of events in experiments 1–5*

Time, h	Experiment 1 Cow 49					Experiment 2 Cow 49					Experiment 3 Cow 020					Experiment 4 Cow 49				Experiment 5 Cow 66			
	RF	LF	RH	LH		RF	LF	RH	LH		RF	LF	RH	LH		RF	LF	RH	LH	RF	LF	RH	LH
0 —	m	m	m	m	b	m		m		b	m	m			b		m	m				m	m
					b			55				60					55				50		
3 —	m			m	b																		
			50																				
—					b•																		
6 —	m	m	m	m	b	m	m	m	m	b	m	m	m	m	b	m	m	m	m	m	m	m	m
					b																		
9 —	m	m	m	m	b																		
					b																		
12 —	m	m	m	m	b	m	m	m	m	b	m	m			m	b							
					b																		
15 —		m	m		b																		
					b																		
18 —	m	m	m	m	b	m			m		b	m	m	m	m	b							
					b																		
21 —	m	m	m	m	b																		
					b																		
24 —	m			m	b	m	m	m	m	b		m	m			b							
					b			60				65											
27 —	m	m	m	m	b																		
					b																		
30 —						m		m		b	m	m	m	m	b								

* Blood samples taken at 15 minute intervals between milkings at 3 and 6 h.
RF, right fore; LF, left fore; RH, right hind; LH, left hind.
m, milk removed from quarter: b, samples of venous blood obtained; ——, water, kg given after milking.

of which had not been milked out at the previous milking, was removed in successive portions of about 200 ml and a sample taken from each portion. Samples of jugular-venous blood were taken, through an in-dwelling polythene cannula, at intervals throughout expts. 1–3. Details of expts. 1–5 are given in Table 2.

In expt. 6, the animals had unrestricted access to water. Samples of jugular-venous blood and of mammary-venous blood were taken simultaneously, by veni puncture, and immediately afterwards the lactating cows were milked and samples of milk taken.

Methods of analysis

Milk samples were analysed for fat by the Gerber method (British Standards Institution, 1955) and for total solids gravimetrically (British Standards Institution, 1951). Lactose was determined by a modification of the method of Hinton & Macara (1927) and chloride by an adaptation of the Volhard method (Davies, 1932). Total nitrogen, non-casein nitrogen and non-protein nitrogen were determined by the method of Rowland (1938) and sodium and potassium by flame photometry, using a Unicam SP 900 flame spectrophotometer. The Δ values of milk and of blood serum were determined using an improved Temple apparatus (Temple, 1937), the thermometer readings being corrected from an NPL certificate. The thermometer was also standardized against sucrose solutions, and the same corrections were obtained. No correction was made for any alterations in the carbon dioxide content of blood or milk between collection and the determination of Δ.

Intake of
50 kg water
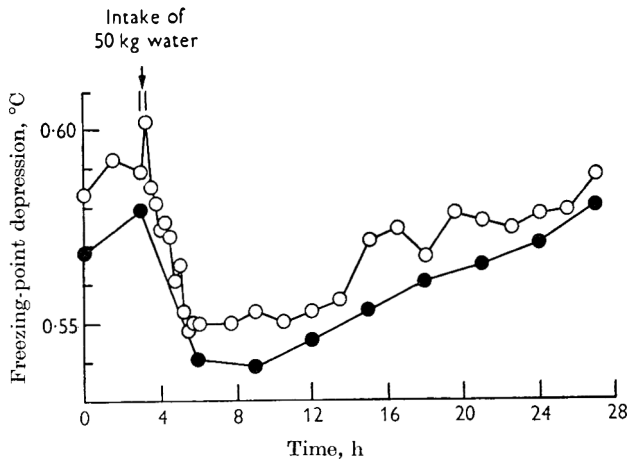
Fig. 1. The time-course of the changes in the freezing-point depression, Δ, of jugular-venous blood serum and of milk in Cow 49 (expt. 1) when access to water was restricted to a single short period in each 24 h; ○, jugular-venous blood serum; ●, milk.

RESULTS

Freezing-point depression of blood and milk

The time-course of changes in the Δ of blood and milk in a cow with access to water for a single short period in each 24 h is illustrated in Fig. 1 using the results of expt. 1. After the ingestion of water the Δ of the blood serum fell steadily over a period of $2\frac{1}{2}$ h, from a peak value of 0·602 to about 0·550. The value steadied at this level for some 5 h and then increased progressively to the original value after 24 h. A similar pattern was observed for milk but the values were consistently lower, by 0·007–0·018 (mean value, 0·011). The mean difference and the variations in the difference from time to time appear to be too large to be accounted for solely by experimental error. Comparable discrepancies between the values for jugular-venous blood serum and milk were observed also in expts. 2 and 3 (Table 3), the mean differences being 0·026 and 0·014, respectively.

The results of the comparison of the Δ values for the blood serum of the jugular vein and of the mammary vein and for milk made in expt. 6 with 4 lactating cows with unrestricted access to water, are given in Table 4. The values for Glee 18 show that under certain conditions the Δ of milk may be higher than that of blood serum of the jugular vein. Invariably, however, the Δ of blood serum from the mammary vein was intermediate between that of blood serum from the jugular vein and that of milk. Moreover, the differences between the values for the blood serum of the

Table 3. *The freezing-point depression* (°C) *of the jugular-venous blood serum and of the milk from the separate quarters of the udder for Cow 49 in expts.* 1 *and* 2 *and Cow* 020 *in expt.* 3

Time, h	Quarter				Blood serum
	Right fore	Left fore	Right hind	Left hind	
		Cow 49, expt. 1			
0	0·567	0·568	0·568	0·569	0·583
3*	0·578	†	†	0·580	0·589
6	0·538	0·540	0·541	0·540	0·550
9	0·537	0·539	0·539	0·539	0·553
12	0·542	0·545	0·546	0·548	0·553
15	†	0·550	0·556	†	0·571
18	0·560	0·559	0·560	0·561	0·567
21	0·564	0·563	0·566	0·568	0·576
24	0·571	†	†	0·571	0·578
27	0·578	0·580	0·581	0·580	0·588
		Cow 49, expt. 2			
0*	0·572	†	0·571	†	0·596
6	0·543	0·540	0·540	0·540	0·561
12	0·550	0·546	0·552	0·548	0·561
18	0·563	†	0·565	†	0·583
24*	0·578	0·577	0·574	0·579	0·614
30	0·534	0·532	0·533	0·534	0·575
		Cow 020, expt. 3			
0*	†	0·599	0·593	†	0·620
6	0·540	0·540	0·538	0·542	0·556
12	0·555	0·553	†	0·558	0·566
18	0·574	0·576	0·574	0·573	0·589
24*	†	0·588	0·586	†	—
30	0·530	0·532	0·530	0·536	0·550

* Water offered immediately after milking (see Table 2 for details).
† Milk not removed from the quarter.

mammary vein and of the jugular vein in the 4 lactating cows of 0·012, 0·009, 0·012 and 0·004 are, on average, measurably higher than the corresponding differences in the 2 dry cows of 0·005 and 0·001 which are probably within the limits of experimental error. It appears that milk formation is associated with a slight alteration in the Δ of blood passing through the mammary gland, the extent of the alteration possibly varying from cow to cow and from time to time. The slight differences observed between the Δ value for milk and blood serum from the mammary vein would be explained by a partial recovery to the original value in the blood during its passage from the mammary gland to the sampling site.

The Δ values of the samples of milk obtained from the separate quarters of the udder in expts. 1, 2 and 3 are given in Table 3. The values for all 4 quarters of an

6-2

udder were similar, both in cow 49 with little or no udder damage and in cow 020 in which there was evidence in 2 quarters of damage due to injury or bacterial infection. Rowland, Aschaffenburg & Veinoglou (1943) have previously reported that mastitis does not affect the Δ of milk unless the secretion from the affected quarter is visibly abnormal. Values similar to those for other quarters were observed also in quarters that had not been milked out at the previous milking. The close agreement obtained is well illustrated by the results from cow 49 (expt. 2). Immediately before the intake of water, the values for the right fore and the right hind quarters were 0·572 and 0·571, respectively, and presumably the values for the milk present in the other

Table 4. *The freezing-point depression (°C) of blood serum of the jugular and mammary veins and, where appropriate, of milk taken simultaneously in 4 lactating and 2 non-lactating Friesian cows with unrestricted access to water (expt. 6)*

	Cow	Jugular-venous blood serum	Mammary-venous blood serum	Milk
Lactating	Begonia 2	0·571	0·559	0·557
	Glee 5	0·576	0·567	0·556
	S III	0·569	0·557	0·551
	Glee 18	0·553	0·557	0·564
Non-lactating	Bride	0·568	0·563	—
	Gay	0·590	0·589	—

Table 5. *The freezing-point depression (°C) of consecutive portions of milk removed from single quarters of the udder at the second milkings in expts. 4 (Cow 49) and 5 (Cow 66) (see below and Table 2 for details)*

	Cow 49		Cow 66	
Portion	Right fore* quarter	Left hind quarter	Right fore* quarter	Left hind quarter
1	0·548	0·551	0·576	0·570
2	0·553	0·556	0·570	0·572
3	0·554	0·562	0·567	0·574
4	0·556	0·560	0·568	0·568
5	0·550	0·557	0·568	0·569
6			0·567	

* Milk not removed from this quarter at the previous milking.

2 quarters were close to these values. Six hours later the values for the 4 quarters were left fore 0·540, right fore 0·543, left hind 0·540, right hind 0·540. Thus, in addition to any osmotic equilibrium maintained between blood serum and milk during milk formation in the cells of the alveoli, the osmotic pressure of milk excreted from the cells and held in the udder and teat cisterns, or in the duct system of the udder, must vary continuously with variations in the osmotic pressure of blood. A more rigorous demonstration of the completeness of this equilibrium throughout the entire udder was obtained in expts. 4 and 5 (Table 5). For some days previously the 2 cows had been offered water for a restricted period of 15 min each day. On the day of experiment, immediately before water was offered, only 3 of the quarters of the udder were milked out. Then 6 h later the milk was removed from the unmilked quarter and from one other quarter, in successive portions of about 200 ml. No obvious differences between the 2 quarters in the Δ observed for the various portions of milk were

obtained, in spite of the fact that the milk left in the quarter not milked out at the previous milking must have had at that time a Δ about 0·040 higher than that of milk secreted towards the end of the 6-h period.

Milk composition

The changes in milk composition associated with changes in Δ observed in expts. 2 and 3 are shown in Fig. 2. The normal milking process removes only 50–75 % of the
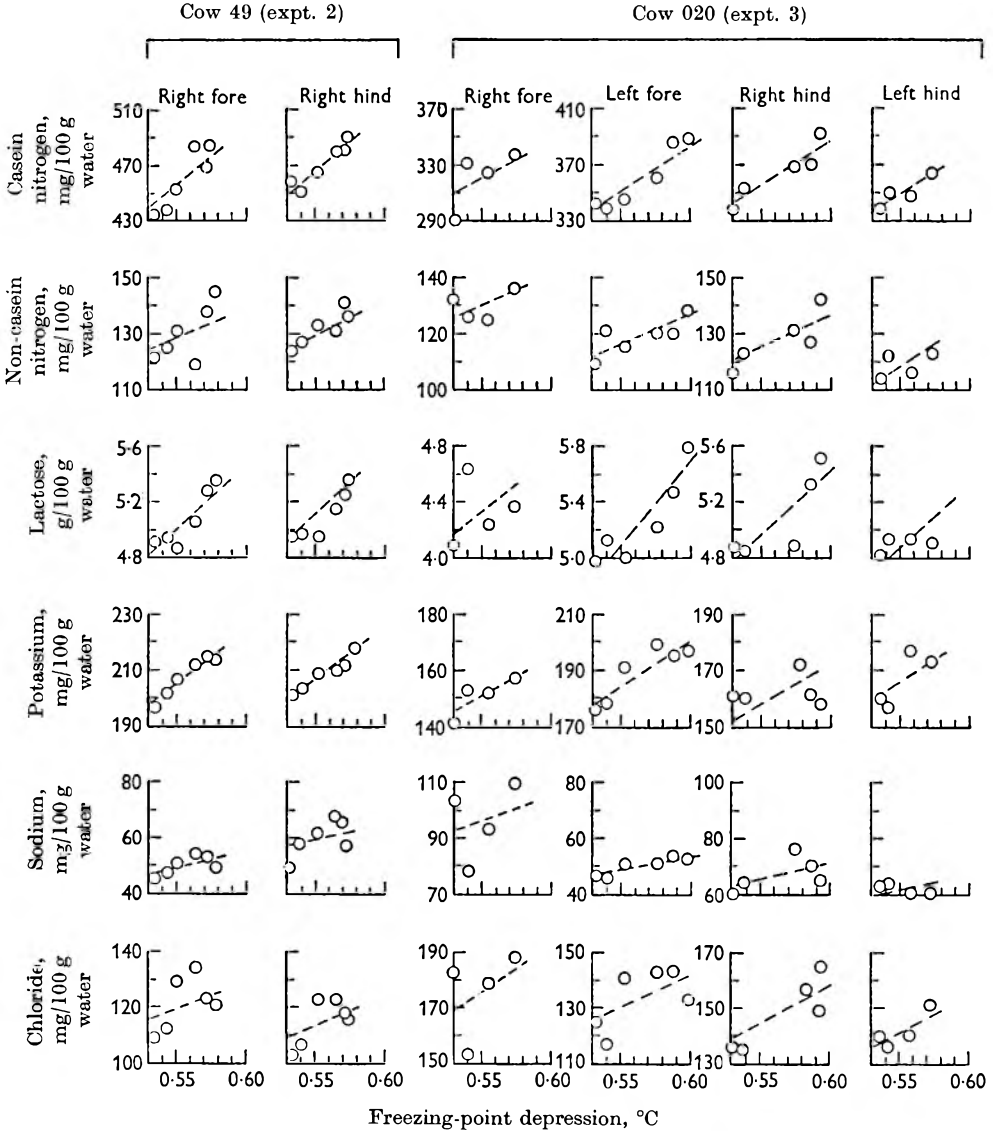
Fig. 2. Variations in the casein nitrogen, non-casein nitrogen, lactose, potassium, sodium and chloride contents of milk with changes in freezing-point depression, Δ. The dotted lines are drawn through the mean for the points on each graph in the direction of the origin. They indicate the lines along which points would regress if the observed changes in milk composition were due solely to a movement of water into or out of the udder as a result of a change in the osmotic pressure of milk.

fat present in the udder at the beginning of a milking (Johannson, 1952). Because the amount of this residual fat varies considerably, particularly with short-interval milkings, the fat content of the milk removed from the udder also varies. The results for fat content have not therefore been reported. Considerable variations in the contents of other milk constituents were observed and, more particularly for the contents of lactose, potassium and casein, the variations were generally in direct proportion to the change in Δ: the concentrations of minor constituents—the non-casein nitrogen fraction and the sodium and chloride—characteristically are more markedly variable from milking to milking than the concentration of lactose, casein and potassium, especially during periods of short-interval milking. The marked irregularities in the relationship between freezing-point depression and the lactose and mineral contents of the right fore quarter of cow 020 (expt. 3) were probably the result of bacterial infection of the quarter.

To study the proportionate changes in milk composition with changes in osmotic pressure, values for casein, lactose, potassium and Δ for each quarter were expressed as a percentage of their mean value. Excluding the results for the right fore quarter of Cow 020, analysis of the combined results of Fig. 2 gave the following regression coefficients with S.E.:

regression coefficient for casein on Δ, $b = 1 \cdot 134 \pm 0 \cdot 0937$;
regression coefficient for lactose on Δ, $b = 0 \cdot 992 \pm 0 \cdot 1267$;
regression coefficient for potassium on Δ, $b = 0 \cdot 761 \pm 0 \cdot 1520$.

Each of these regression coefficients is highly significant ($P < 0 \cdot 01$) and none differs significantly ($P > 0 \cdot 05$) from $1 \cdot 0$. Analysis of variance showed that regression coefficients calculated from the values for single quarters do not differ significantly ($P > 0 \cdot 05$) from those obtained for the combined results. This information is consistent with a direct proportionality between Δ and the concentrations of casein, lactose or potassium in milk.

<div align="center">DISCUSSION</div>

Milk secretion, the combined processes of the formation of milk within the alveolar cells and its excretion into the alveolar lumen and the ducts and cisterns of the udder (Cowie *et al.* 1951), is continuous, but under normal management conditions milk is removed from the udder only at intervals of several hours. Our results have shown that, in addition to any regulation of osmotic activity of milk during its formation, the osmotic pressure of the secreted milk held within the udder alters in association with any change in the osmotic pressure of blood. Thus, though for many purposes the assumption is made that milk removed from the udder by the milking process is that secreted by the alveolar cells, in physiological terms this is not strictly correct. Indeed, under environmental conditions which cause slight variations in the osmotic pressure of body fluids, a net exchange of water or of water-soluble constituents between blood and milk must occur in the interval between the secretion of milk and its removal from the mammary gland. The broadly linear relationships observed between Δ and the concentrations in milk of lactose casein and potassium are consistent with a movement of water into, or out of the udder, in response to any change in osmotic pressure. From the information available, however, the possibility cannot

be excluded that in addition to the movement of water there may be a small reverse movement of water-soluble constituents, more particularly in quarters of the udder that have been damaged by bacterial infection or physical injury. Lactose has been reported to occur in small quantities in the blood and urine of milking cows (Hayashi, 1960).

Though the Δ values of milk and blood serum of the jugular vein are invariably similar, they are not identical. Quantitatively, the contribution to osmotic pressure of materials taken up by the mammary gland from its arterial blood supply, will not necessarily equal the contributions of the synthesized milk constituents and of the materials returned from the gland to the venous blood. Any difference must tend to alter the osmotic pressure of body fluids within the immediate locality of the mammary gland.

From a study of the interrelationships of lactose and potassium in milk, Rook & Wood (1959) concluded that the water of milk, as secreted by the alveolar cells, arises in two ways. Part is initially present in the secretory cell as water of the potassium-rich intracellular fluid. The remainder arises because the synthesis within the cell of lactose, together with protein and fat, is coupled with a movement of water from the blood or other extracellular fluid into the cell to maintain osmotic equilibrium. This movement, they suggested, continues until there is a given volume of fluid within the secretory cell, when the contents are expelled. The water associated with the potassium present initially in the cells, and consequently the amount of lactose and associated water required to be secreted into the cell to achieve the given volume, would therefore be expected to vary with osmotic pressure. A change in osmotic pressure within the body should thus alter the ratio of lactose to potassium in the expelled fluid. A comparison was, therefore, made of the lactose to potassium ratios observed with Cow 49 (expt. 2) and Cow 020 (expt. 3) for the milk secreted in the 6-h interval immediately before water was offered, when the Δ of the blood was continuously above the average value, and for milk secreted over the 4th to the 6th hour after water was offered, when the Δ was continuously below average. Consistent with the above hypothesis, the lactose (g/100 g milk $\times$ 10^2) to potassium (mg/100 g milk) ratio of milk secreted by a single quarter during a period of high osmotic pressure was invariably higher than that secreted during a period of low osmotic pressure, the mean ratios, with S.E., being $2\cdot48 \pm 0\cdot025$ and $2\cdot38 \pm 0\cdot034$, respectively.

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A method of maintaining a reference flora of constant bacteriological composition

By B. REITER, T. F. FRYER and M. ELISABETH SHARPE

National Institute for Research in Dairying, Shinfield, Reading

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SUMMARY. A method is described by which the microbial flora of a raw milk sample was reconstituted on different occasions. After segregation on selective media into groups consisting of lactic acid bacteria, Gram-negative rods including coli-aerogenes, staphylococci, streptococci, and microbacteria, and propagation by replica plate techniques on optimal media, suspensions of these groups of organisms were then added in approximately the original proportions to 40 gal batches of heated milk of low bacterial count. When this was repeated on 18 different occasions over a period of 11 weeks, the milk flora was found to be almost identical with the original one.

INTRODUCTION

In dairy research, bacteriological experiments particularly long-term ones, are often confused because of the variability of the milk flora. Whilst it would be desirable to establish and maintain a mixed flora of constant composition, the isolation of large numbers of strains from solid media and their preservation by continuous sub-culturing or lyophilization is impracticable.

By means of selective media many of the commonly occurring microbial components of milk can be segregated into different groups of organisms so that minority as well as majority groups can be isolated and enumerated. Replication of colonies of these groups by the replica plating technique of Lederberg & Lederberg (1952) or modifications of it, appeared to be a possible method of maintaining a milk flora. This could then be mixed together and reconstituted in heat-treated milk by the addition of suspensions in their approximate original proportions to form a 'reference flora'. The following is a description of the use of such a technique. Several reference floras are being used to investigate the effects of bacteria on the flavour of Cheddar cheese made with the aseptic vat technique of Mabbitt, Chapman & Sharpe (1959).

METHODS

Enumeration and segregation of different groups of micro-organisms present in raw milk

Serial dilutions of milk samples in 0·1 % peptone water were plated on the surface of various selective and non-selective media for the isolation and enumeration of specific groups of organisms.

Gram-negative rods

Pseudomonads, Achromobacter and coli-aerogenes were isolated on nutrient agar, containing 1 pt crystal violet in 500000 pts agar medium (NACV).

Coli-aerogenes

The violet red bile agar (VRBA) recommended by American Public Health Association (1953) was used, the plates being incubated at 30 °C for 24 h. An examination of single colonies of the total Gram-negative flora isolated on the NACV medium revealed a much higher count of coli-aerogenes organisms than found on the VRBA. This may have been due either to the use of surface plates of the latter medium, and not deep plates as recommended, or because the VRBA is more inhibitory.

Staphylococci and micrococci

Salt mannitol agar (SMA) of Chapman (1945) was used.

Streptococci

The thallous acetate tetrazolium (TlTG) medium of Barnes (1956) was used. To isolate group D streptococci only, the plates were incubated at 45 °C for 3 days as suggested by Franklin & Sharpe (1963) so that the growth of other streptococci was inhibited.

Lactobacilli, leuconostocs and pediococci

Acetate agar (TCA) of Rogosa, Mitchell & Wiseman (1951) was used, plates being incubated in an atmosphere of 90% H_2 + 10% CO_2.

Microbacteria

These bacteria were not present in the milks so far examined in sufficient numbers to be isolated as a majority flora and suitable selective media for their isolation have not yet been devised. However, heat treatment of the milk at 155 °F for 15 sec resulted in destruction of a large part of the other bacteria so that after plating on yeast glucose agar (YDA) or on nutrient agar (NA) the microbacteria in these samples could be distinguished readily as yellow or orange pigmented colonies. These colonies were enumerated, picked off, purified and for replica method I (see below) nutrient broth cultures of each strain, adjusted to the same optical density, were mixed in equal proportions. Serial dilutions of the mixtures were plated on nutrient agar and plates replicated in the same way as with the selective media.

Total non-lactic acid bacteria

Milk samples were plated on nutrient agar (NA) to obtain a total colony count of the organisms not requiring carbohydrate for growth, i.e. Gram-negative rods, staphylococci and microbacteria, to obtain a comparison of the number of these organisms on selective and non-selective media.

For the particular purpose of this work aerobic spore formers were not isolated for

incorporation in the reference flora, but colonies could be picked from a non-selective medium and treated in the same way as the microbacteria.

All plates were incubated for 5 days at 30° C unless otherwise specified.

Replication of groups of organisms

Replica method I

Suitable plates, containing well-spaced colonies, for each group of organisms were replicated on the appropriate selective medium using the technique of Lederberg & Lederberg (1952).

Organisms were replicated on 2 occasions on the selective media to get rid of any micro-colonies or inactive viable cells of organisms not belonging to a particular group which might multiply when replicated on non-selective media. Organisms not belonging to the desired group, and which could be detected by their colony appearance, such as colonies of aerobic spore formers, were sometimes found on first isolation on the selective media. Such colonies were removed by cutting them out of the agar and then replicating the remaining colonies. Further replications were made weekly, alternating selective and non-selective media to retain vigorous growth and exclude contaminants. As non-selective media, nutrient agar was used for the Gram-negative rods, staphylococci and microbacteria, YDA for streptococci, and MRS agar (de Man, Rogosa & Sharpe, 1960), for the lactobacilli, pediococci and leuconostocs.

The identity of many of the colonies belonging to each group maintained as a reference flora was checked by picking off and subjecting to accepted identification tests.

When this method was used to replicate a reference flora over a period of several months it had the disadvantage that after many replications the colonies gradually increased in size and became merged together, or became smaller and died out so that the original flora was no longer replicated. Accordingly, although this method was suitable for short term experiments which did not require more than a few replications, another method was devised for long term experiments.

Replica method II

A multiple inoculator based on those described by Roberts (1959) and Weinberg & Arthur (1960) was used to replicate growth. It consisted of a circular perspex base with 45 stainless steel blunt ended pins punched into it in rows. The number of these could of course be varied. A detachable stainless steel handle was screwed on to a projection from the top surface of the perspex base. This replicator can be made cheaply and readily and it was convenient to have a set of them. They were sterilized by flaming after immersing in alcohol several times.

The points of the replicator were pressed lightly on to the sterile surface of the relevant non-selective medium in order to indent the agar in a predetermined pattern. Strains of the group of organisms to be replicated had previously been obtained by plating out the raw milk sample on the selective media as for replica method I and picking each colony off the plate into optimal broth media. After purification a broth culture of each strain was spot inoculated with the tip of a sterile wire into one of the indentations in the agar, so that after incubation single colonies of each strain developed in a pattern corresponding to that of the replicator pins. To transfer the

growth, the replicator was touched on to the plate so that each pin became inoculated with a colony, and was then touched on to a plate of sterile medium.

Although initially more laborious, this method had the advantage that the colonies did not increase in size after a number of transfers, a constant area of growth on the tip of the pin being consistently transferred; also if one colony failed to grow its absence was readily noticed in the orderly arrangement of colonies. In addition, by examining some of the characteristics of the strains to be replicated before arranging them on the plate, subdivisions could be made within a group, and similar strains placed together.

For work concerned with the microflora of cheese, lactic acid bacteria were not only divided into genera but also into homo- and heterofermentative strains; staphylococci were divided into coagulase and mannitol positive and negative strains and Gram-negative rods into genera and lipolytic and non-lipolytic strains. Subgroups could be omitted from the experiment as desired by cutting out the appropriate part of the plate and replicating the remainder.

Forty-five was a convenient number of colonies to work with, although with some of the groups of organisms the numbers were less than this. For maintenance, the colonies were replicated in duplicate on non-selective media.

Reproduction of raw milk flora (reference flora)

Using the appropriate replica method to obtain growth of the organisms, a saline (0·85 %) suspension containing a mixture of the different groups of organisms in numbers and proportions calculated to reproduce the original flora was prepared as follows. Each group was replicated on its respective non-selective medium, using more than one plate if necessary to obtain a sufficient density of cells, for 18–24 h at 30 °C. Growth was washed off with 10 ml saline. All suspensions were adjusted on the Hilger and Watts 'Biochemical' absorptiometer using filter No. 58 to give an optical density of 0·40. The number of organisms of each group corresponding to this optical density was determined previously. From this the volume of each cell suspension to be added to the chosen volume of milk (in this case 40 gal) to obtain the original number of viable cells/ml milk was calculated. These volumes were mixed together and added to the milk which had been heat-treated at 161 °F for 17 sec. This constituted the reference milk. In addition to the reference flora such a milk also contained small numbers of aerobic spore formers, which were originally present in the milk as spores and were not destroyed by the heat treatment.

RESULTS

Table 1 shows the numbers of the different groups of organisms found in a raw milk and in 18 milks to which the reference flora from the raw milk had been added over a period of 11 weeks. The total count of non-lactic acid bacteria in the raw milk on the non-selective medium was 50×10^4/ml whilst the total additive count of these organisms on the selective media was 33×10^4/ml, indicating little difference. It is evident that with the reference milks a similar flora was obtained on each occasion. Such variations as occurred were usually within the limits to be expected on duplicate plating of a single sample of milk by the usual methods. With

Table 1. *The numbers of organisms of the different groups found in a raw milk and in 18 milks to which the raw milk reference flora had been added*

No. of organisms $\times 10^{-3}$/ml milk

Group of organisms	Original raw milk flora	Reference flora milk number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Lactic acid bacteria Lactobacilli Leuconostocs	0·25	0·1	0·27	0·32	0·35	0·2	0·2	0·27	0·19	0·19	0·3	—	0·5	0·29	0·37	0·37	0·36	0·
Non-lactic acid bacteria isolated selectively																		
Gram-negative rods, total	20	30	25	24	42	23	36	42	19	37·5	—	44	42	33·5	41	31	29	39
Gram-negative rods, coli-aerogenes	8	18	9·8	7·2	16	8	5·5	18	9·5	14·5	—	28	10	21	—	12	6	20
Staphylococci and micrococci, total	8	15	18	16	7	5	7	3·6	5·1	4·1	4	3·5	—	11	18	4·3	7	3·
Staphylococci and micrococci, mannitol positive	3	.	6	.	3·5	3	2·5	0·8	2·1	1·75	3·9	1·3	—	5	1·1	3·3	4	1·
Microbacteria	5	.	4·2	14	1	1·5	6	3	2·5	2·25	2	2	5·5	5	4	3·5	—	4
Non-lactic acid bacteria isolated on non-selective medium	50	40	142	83·5	46	35	46	50	38·5	42·5	66	61	45	48·5	60	30	29	40

— Group not included in flora on that occasion.

. No count made.

* Not included in total non-lactic acid bacteria, as constitute part of the flora of that group.

this particular milk (Table 1), streptococci were only recovered on a non-selective medium (YDA) incubated at 37 °C, and not on the TlTG medium as this was incubated at 45 °C. These organisms proved to belong to serological group B, and were not incorporated in the reference flora as they were irrelevant to the particular

Table 2. *Comparison of counts of micro-organisms in a sample of raw milk with counts in a reference flora milk reconstituted on different occasions*

All counts expressed as log (number of organisms per ml milk $\times 10^{-3}$)

		Reference flora milk samples				
Group of organisms	Original raw milk flora (1 sample) x_0	No. of samples n	Mean $\bar{x}$	Standard error of mean $s_{\bar{x}}$	Difference, $\Delta = \bar{x} - x_0$	Standard error of difference, s_Δ
Non-lactic acid bacteria isolated selectively:						
Gram-negative rods, total	1·301	17	1·523	+0·028	+0·220†	±0·118
Gram-negative rods, coli-aerogenes	0·903	15	1·085	±0·055	+0·182 NS	±0·220
Staphylococci and micrococci, total	0·903	17	0·820	±0·065	−0·083 NS	±0·275
Staphylococci and micrococci, mannitol positive	0·477	15	0·402	±0·064	−0·075 NS	±0·258
Microbacteria	0·699	16	0·514	±0·068	−0·185 NS	±0·278
Total non-lactic acid bacteria	1·519	14	1·656	±0·027	+0·137 NS	±0·104
Non-lactic acid bacteria isolated on non-selective medium:	1·699	18	1·687	±0·039	−0·012 NS	±0·168
Lactic acid bacteria Lactobacilli Leuconostocs	−0·602	17	−0·582	±0·043	±0·020 NS	±0·183

† Significant at the 10 % level ($P < 0.1$). NS, non-significant ($P > 0.1$).

Table 3. *Identification of strains of organisms isolated on different selective media from a raw milk sample and later constituting a reference flora*

Group of organisms	Medium of isolation	No. of strains identified	No. of strains examined/total replicated
Gram-negative rods			
Pseudomonads	NACV	30	
Achromobacter	NACV	6	
Enterobacteria:			
lactose positive	NACV	9	88/220
Coli-aerogenes	NACV and VRBA	36	
Unclassified	NACV	7	
Staphylococci			
Coagulase and mannitol positive	SMA	6	
Coagulase and mannitol negative	SMA	21	27/88
Lactic acid bacteria			
Lactobacilli (homofermentative)	TCA	7	
Leuconostocs	TCA	11	18/24
Streptococci (group B)	YDA	28	28/63
Microbacteria			
Lemon pigmented	NA	24	
Orange pigmented	NA	2	26/26

NACV, Nutrient agar crystal violet agar; VRBA, violet red bile agar; SMA, salt mannitol agar; TCA, acetate agar; YDA, yeast dextrose agar; NA, nutrient agar.

experiment. In Table 2 a statistical analysis of these results is given. The difference, Δ, between the mean of the numbers of any bacterial group in the reconstituted flora of the milks and the number of that group in the original sample was significant only in the case of the Gram-negative rods and then only at the 10% probability level. The sum of the counts of groups of non-lactic acid bacteria in the original raw milk sample. 1·519, did not differ significantly from the corresponding count, 1·699, obtained by isolating on a non-selective medium. Similar agreement between total non-lactic acid bacteria counts determined on selective and non-selective media was shown for the milk samples with reconstituted flora.

When identification of a number of the components in a reference flora was undertaken (Table 3), it was found that such a flora did in fact include a number of different species isolated in each medium. Even though the tests used were usually only those sufficient to allocate organisms to a genus or occasionally to species level, it was evident that a variety of species was present. More extensive physiological characterization would no doubt reveal further differentiation between some of the strains.

DISCUSSION

The techniques described in this paper aimed to preserve the proportions between the groups of organisms occurring naturally in raw milk and thus make it possible to work with a constant microflora over an extended period or to vary the proportions or numbers of this flora as desired. Whilst it was our purpose to restore the original milk organisms maintained as a reference flora, the accuracy of the method is limited by the initial method of isolation of the organisms on selective media. It is well known that more sensitive strains of any species may be inhibited on selective media often designed to suppress near related species and it is therefore unlikely that a 100% recovery would be obtained. However, there is a good agreement between the sum of total numbers of non-lactic and bacterial colonies found on the selective media compared with those found on the non-selective medium (Table 2). In addition even a non-selective medium will not support the growth of all bacteria occurring originally in the milk, particularly if incubated at any one temperature only. The agreement between the original and the reconstituted reference flora in 40 gal of milk, is therefore very satisfactory when considered within these limitations.

The replica methods described are unsuitable for obtaining a representative flora from heat-treated milk because bacterial cells which have received sublethal heat treatment are often rendered sensitive to inhibitory substances present in selective media so that some of them, although viable, will not multiply (Reiter, Fewins, Fryer & Sharpe, 1964). The reference milk flora also differ from those of normal raw milk in that the phase of growth of the various bacterial groups may be different. Any attempts to age the milk may result in a distortion of the proportions of the different groups.

The reference flora can be modified according to the experimental requirements by changing the total number of organisms or proportions between the groups of organisms, or omission of one or more groups.

Whilst this paper describes the particular application of this technique to reconstitution of raw milk and its use in studies of Cheddar cheese, it could also be used

for other work where a standard milk flora is required over a period of time. In addition, such a reference flora technique is not restricted to milk, as an appropriate flora of any mixed population could be isolated, maintained and reconstituted provided that suitable media were available.

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Reviews of the progress of dairy science

Section E. Diseases of dairy cattle. Salmonella infection in cattle

By E. A. GIBSON

Veterinary Investigation Centre, Norwich

CONTENTS

Although this journal's biennial reviews on milk-borne diseases have included references to salmonella infection of cattle, this is the first occasion on which the subject has been reviewed on its own. It is therefore appropriate to give a brief survey of the history of the disease before considering the additional material published during the 4 years under review (1960–63 inclusive). In addition, each section of the review is prefaced by a brief summary of work published before 1960.

DEFINITIONS, INTRODUCTION AND HISTORICAL SURVEY

The genus *Salmonella* is one of the several genera and groups which together comprise the family *Enterobacteriaceae*. Edwards & Ewing (1962) described this family as being composed of a large number of interrelated types displaying almost every conceivable combination of biochemical characteristics compatible with the definition of the family. They added that these types form a continuous series that is not readily divisible into distinct groups, although it is possible to distinguish dense centres of population with similar biochemical properties. These centres have been

used as the basis of the groups and genera, such as *Salmonella*, *Proteus* and *Escherichia*, that are generally recognized within this family, but the authors comment that although such divisions are desirable for practical purposes it must be remembered that they are purely artificial, that many intermediate strains exist, and that even typical members of the various groups commonly show inter-group relationships in the form of shared biochemical or serological properties.

Because of these interrelationships it has been necessary to modify the definition of the genus *Salmonella* from time to time in order to accommodate newly discovered organisms with properties intermediate to those of other genera, as well as to accommodate various advances in bacteriological technique. Thus successive authoritative definitions have been offered by White (1929), the Salmonella Subcommittee of the International Association of Microbiologists (1934, 1949), Kauffmann (1954, 1961) and the Subcommittee on the Taxonomy of the Enterobacteriaceae (Report, 1963). It is self-evident that further modifications may be required from time to time as new strains are isolated and new techniques are evolved. However, for the purpose of this review it is sufficient to state that the genus *Salmonella* is defined elsewhere on the basis of its biochemical and serological characteristics and to point out that all the known members of the genus are believed to be pathogenic for man or animals, or both (Kauffmann, 1954).

In this review the term 'serotype' will be used to refer to the constituent members of the genus *Salmonella*, chiefly to avoid any possible confusion between references to species of salmonellae and the host species in which they occur.

Cattle of all ages may become infected with various salmonellae which may cause either clinical disease or sub-clinical infection. In this review the term 'salmonellosis' will be restricted to clinical disease and the term 'salmonella infection' to infection without clinical manifestation. The term 'cattle' obviously refers chiefly to *Bos taurus*, but references to infection in other bovine species will be included when they are of interest. The author's own use of the word 'calf' will refer to cattle up to 6 months old, but as this definition is not generally accepted it must not be assumed that citations from other authors follow this definition.

Clinical salmonellosis is more common in calves than in older cattle. In the latter the disease is usually sporadic. Affected animals typically show clinical signs of septicaemia followed by diarrhoea or dysentery, and death commonly occurs within 2–4 days of the first observed signs of illness. Some animals show less acute signs, and a gradation can be traced from cases showing the typical signs outlined above to those in which infection is inapparent.

Adult animals that recover from the clinical disease tend to continue to excrete the causal organism in their faeces. This is especially so with *S. dublin* infection, in which excretion may persist for years, if not for life. Cattle in this state will be referred to as 'constant excretors'. Other animals become constant excretors as a result of the mild or subclinical infections mentioned above. Others show only intermittent excretion of the organism, and the term 'active carriers' will be used as comprising both constant and intermittent excretors. Other cattle acquire a latent infection which may be disclosed by excretion of the organism when the animal is put under stress by, for example, some intercurrent disease. These will be referred to as 'latent carriers'.

Adult or yearling cattle showing any of these manifestations of salmonella infection can act as a source of infection for calves, which may be exposed to infection at any age from birth upwards, or *in utero* (Field, 1948; Rasch, 1953).

In calves the disease tends to spread rapidly to affect a high proportion of those at risk. The clinical picture is more variable than in older cattle, both in its severity and in the nature of the clinical signs exhibited. This variability can be observed not only between individual cases in a given outbreak but also from one outbreak to another. Thus pneumonia is not uncommonly present, and may sometimes dominate the clinical picture. Arthritis is also a not uncommon feature of the disease in calves. Usually the course of the disease is more prolonged than in older cattle, often resulting in chronic unthriftiness.

Bovine salmonellosis was recognized on the mainland of Europe and elsewhere, both in calves and in older cattle, many years earlier than in Britain. Savage (1918, 1920), Henning (1939, 1953a) and Gibson (1958, 1961) have provided reviews of its emergence as a separate entity.

As would be expected, the early history of the disease is confused because at that time the genus *Salmonella* was inadequately defined. Few of its constituent serotypes had been recognized, and these were commonly confused with members of other genera. The earlier reports must therefore be interpreted with caution, but even so it is apparent that bovine salmonellosis has existed as a clinical entity for many years and that the organisms isolated by the earlier workers were probably the same as those found today.

In calves, a form of diarrhoea or *kälberruhr*, with clinical signs closely resembling those of salmonellosis, has been recognized on the mainland of Europe for at least 150 years. It appears that Obich (1865) was the first to regard the disease as due to an infectious agent, but it was not studied bacteriologically until Jensen (1891, 1893) investigated an outbreak in Denmark and isolated a coliform bacillus from the viscera of affected calves. Jensen was unable to distinguish the organism morphologically or culturally from strains of *Bacterium coli* (now renamed *Escherichia coli*) isolated from the intestines of normal calves, but found that unlike *B. coli* it was pathogenic for newly born calves and reproduced the clinical disease when fed to them in milk. Similar outbreaks were investigated in Holland by Thomassen (1897) and Poels (1899). Thomassen isolated an organism which he named 'pseudo-typhoid bacillus', thus indicating a relationship with the 'typhoid bacillus' (now *S. typhi*) which Gaffkey (1884) had recently isolated from typhoid fever of man. Poels isolated an organism that he named 'pseudo-coli bacillus'. Jensen (1903) used the term *Bacillus paracoli*, or 'paracolon bacillus' for the organism isolated from the Danish outbreaks, but many of the strains given this name were later shown, with increasing exactitude, to be salmonellae (Titze & Weichsel, 1908; Uhlenhuth & Hubener, 1913; Jensen, 1913; Christiansen, 1915). Most appeared to be *S. enteritidis* but others resembled *S. paratyphi* B (Jensen, 1913). Other reports incriminating salmonella-like organisms as the cause of septicaemia, diarrhoea or pneumonia in young calves were published by a number of workers, including Riemer (1908), Schmitt (1908), Ledschbor (1909), Zeller (1909), Winzer (1911), Miessner & Kohlstock (1912), Luxwolda (1913), Christiansen (1914a), Warnecke (1914), Douma (1916) and Meyer, Traum & Roadhouse (1916).

The first report of salmonellosis in adult cattle was provided by Mohler & Buckley (1902), who described an outbreak in the United States due to an organism resembling *S. enteritidis*. Outbreaks in adult cattle were later reported from Europe by Miessner & Kohlstock (1912), Bugge & Dierks (1922), Lütje (1926), Lehr (1927) and Bourmer & Doetsch (1928). This, however, was before White (1930) established the separate identity of *S. dublin* by distinguishing it serologically from *S. enteritidis*, which resembles it closely both biochemically and serologically. In passing, it is of interest to note that both strains studied by White were of human origin. One, which was isolated in Dublin and so gave its name to the serotype, was from a fatal kidney infection and the other was from a case of meningitis in a child. Smith & Scott (1930), who reported a further 3 cases of *S. dublin* infection in man, also re-examined 38 strains of *Salmonella* isolated some time previously by other workers. Four strains, isolated from man and previously regarded as *S. enteritidis*, were found to be *S. dublin*, as were 6 strains of 'B. paracoli' isolated in Copenhagen from cases of calf dysentery. Similarly, Rajagopalan (1938) reported that strains isolated at Lahore from a septicaemic disease of calves (Shirlaw, 1935) and previously regarded as *S. enteritidis* were found, on re-examination, to be *S. dublin*.

Similar confusion existed between *S. paratyphi* B and *S. typhimurium*. Savage (1909) had concluded that these 2 organisms, which he referred to as 'B. paratyphosus B' and 'bacilli of the aertryke type', were serologically distinct, but this view was not accepted by many workers on the continent of Europe until about 1920 (Savage & White, 1925). However, when White (1926) re-examined a number of strains isolated in Copenhagen from cases of salmonellosis in calves, and described by Christiansen (1914b) as 'B. paratyphosus B (Schottmüller)' (i.e. *S. paratyphi* B), he found that all were typical strains of *S. typhimurium*.

When the genus *Salmonella* became more clearly defined it was apparent that the serotype most commonly associated with bovine salmonellosis in those parts of Europe in which the disease was endemic was *S. dublin*, a serotype that can be regarded as showing a strong host-specificity for cattle. *S. typhimurium* was next in order of frequency, both in calves and in older cattle, but other salmonellae seldom caused clinical disease. Thus in Germany, Gröger (1936) examined 300 calves in which salmonellosis was suspected. He isolated 59 strains of salmonellae and found that all were *S. dublin*. Bartel (1938a) stated that 'the kiel type of *S. enteritidis*' (i.e. *S. dublin*) was predominant in north, east and west Germany, where bovine salmonellosis was widespread. Few cases occurred in south Germany, and these were due chiefly to *S. typhimurium*. He also reported (Bartel, 1938b) that of 1087 strains of salmonellae isolated from calves in 1937, 89·8% were *S. dublin* and 8·6% *S. typhimurium*. The corresponding findings for 253 strains from older cattle were 65·5 and 31%, respectively. Lütje (1939) reported similar figures from Germany, as did Röhrer & Winand (1941) and Pohl (1942).

A similar preponderance of *S. dublin* infection has been reported from other European countries, including Holland (Clarenburg & Vink, 1949), Denmark (Hansen, 1947; Müller, 1954) and Italy (Strozzi, 1934). Similar findings have been obtained in certain areas of the British Isles, in parts of south Wales (Field, 1948, 1949a, b; Gibson, 1958); Northern Ireland (Murdock & Gordon, 1953); Eire (Mullaney, 1949); and in parts of south-west England (Grunsell & Osborne, 1948).

A high incidence of *S. dublin* infection has also been reported from countries in other continents, e.g. South Africa (Henning, 1939); Venezuela (Gallo, 1939); and Brazil (Penha & D'Apice, 1946). Reports of its presence have come from several other countries, including India (Rajagopalan, 1938); Kenya (Shirlaw, 1959); Israel (Ben-David, 1953); Nigeria (Collard & Sen, 1956); and Australia (Stewart & Hayston, 1941). The position in North America is of some interest. Edwards, Bruner & Moran (1948) reported a low incidence of bovine salmonellosis in the United States and attributed this to the absence of *S. dublin* from the greater part of the country. They found that only 10 of 61 strains isolated from cattle were *S. dublin*, and that all 10 were from California. They commented that both in cattle and in other animals, *S. dublin* appeared to be confined to the west of the Rocky Mountains. Moran (1959) also noted an absence of *S. dublin*. Bovine salmonellosis also appears to be uncommon in Canada, although both *S. dublin* and *S. typhimurium* have been recorded (Schofield, 1946). In New Zealand *S. dublin* has been isolated from man but appears to be absent from cattle, and 412 of 416 bovine strains of salmonellae were found to be *S. typhimurium* (Salisbury, 1958).

Summing up the geographical distribution of salmonellae in cattle, it is seen that *S. dublin*, which possesses a considerable degree of host-adaptation for cattle, is widely distributed and presents a major problem in a number of countries. *S. typhimurium*, which exhibits a complete lack of host specificity, is usually also present— but with a lower incidence—in any area in which *S. dublin* occurs. If *S. dublin* is absent, then *S. typhimurium* usually constitutes the chief salmonella infection of cattle.

As would be expected, a wide variety of other salmonellae has also been isolated from cattle. Edwards *et al.* (1948) reported 13, and Buxton (1957*b*), in a list that did not claim to be exhaustive, referred to 28. McAnulty (1958) examined zebu cattle in Uganda and isolated 25 serotypes, including 5 new ones.

It appears that the presence of serotypes other than *S. dublin* and *S. typhimurium* is usually due to contamination of feedingstuffs (Gray, Lewis & Gorrie, 1958) or water (Holz, 1956) or to cross-infection from other mammals, or from birds or reptiles (McAnulty, 1958). Such infections are commonly subclinical and seldom establish themselves in a herd. Some authors, however, have noted with concern an increased incidence of infection with the less common serotypes (Müller, 1957).

The existing literature on other aspects of salmonella infection in cattle is briefly surveyed in each section of this review. At this point it will suffice to say that the subject has attracted considerable interest in recent years, not only because of the economic importance of the disease to the livestock industry, but also because of the importance of salmonellae as a cause of food-poisoning and other types of illness in man, an aspect that was ably reviewed by Buxton (1957*a*). Attention must also be drawn to the excellent general reviews provided by Buxton (1957*b*) and by Field (1959).

AETIOLOGY

Causal organism

As seen from the introduction, work published before 1960 clearly showed that *S. dublin* was the predominant serotype in those areas in which salmonellosis was endemic in cattle. Further evidence on this point was published during the period

under review. Some came from the traditional areas of endemic bovine salmonellosis. Thus in Holland, Kampelmacher, Guinée & Clarenburg (1962) confirmed that *S. dublin* was still the predominant serotype in cattle, accounting for 92% of the isolates. They noted, however, that the proportion of *S. typhimurium* among the isolates from calves had increased during recent years and that this serotype was the only one found in routine examinations of mesenteric lymph nodes from clinically normal calves. Dijkstra (1961) also described *S. dublin* as the predominant serotype in Friesland, Holland, both in calves and in adult cows. Another report from Holland (van Ulsen, 1960) recorded the isolation of *S. dublin* from 113 (1·2%) of 9723 bovine foetuses examined between 1950 and 1959. Liebermann, Müller & Heinke (1960) found a similar incidence of *S. dublin* in bovine foetuses examined in Mecklenburg, Poland, when they isolated this serotype from two (1·1%) of 189 specimens. Uzięblo (1961) reported 2 outbreaks of clinical *S. dublin* infection in adult cattle on the Baltic coast of Poland—an area in which he considered the disease to be endemic. Meuszynski (1962) also emphasized the importance of *S. dublin* infection in Poland. Bulling (1961) provided similar evidence from Lower Saxony, Germany, another traditional area of bovine salmonellosis. *S. dublin* accounted for 92% of all the isolates from calves and *S. typhimurium* for 5%. The figures for isolates from older cattle were 86 and 10%, respectively. Eighteen other serotypes were isolated. Also in Germany, Stoll (1960) cultured calf livers showing miliary necrosis. He isolated *S. dublin* from 41 (40%) of the 103 examined. Another 58 yielded other bacterial growths, but none of these was a *Salmonella*.

In Britain, Gibson (1961) listed the isolations of salmonellae obtained from calf material at Veterinary Investigation Centres in England and Wales from 1958 to 1960 inclusive and confirmed that *S. dublin* was the dominant serotype. Of the 918 strains isolated in this period, 573 (62%) were *S. dublin* and 313 (34%) *S. typhimurium*. The other 32 strains comprised 12 serotypes. However, in contrast to this, Anderson, Galbraith & Taylor (1961) found *S. typhimurium* to be the dominant serotype when they examined British calves at slaughter. Seventy-seven (15%) of 511 calves yielded *S. typhimurium* whereas only 2 gave *S. dublin* and 1 *S. binza*. This, however, was undoubtedly due to a build-up of infection between the farm and the slaughter point in which there was probably a selective action favouring *S. typhimurium*. Amor & Hopkins (1962) described 3 clinical cases of *S. dublin* infection in adult cows in one herd in south-west England (Devon). Their suggestion that the disease might be rare in adult cattle was followed by letters from Fincham (1962) and Gibson (1962) briefly reviewing the literature on this point. Whitty (1960) gave a clinical description of salmonellosis in Eire, as did Hartigan (1960) who confirmed that the disease was endemic in certain districts.

Further indications of the importance of *S. dublin* infection in cattle came from Russia, where Zagaevskii (1962) found that 70 (56%) of 124 strains isolated from cattle were *S. dublin*. Twenty-four (19%) were *S. typhimurium* and 8 *S. enteritidis*. In Belgium, Thomas (1961) found that *S. dublin* and *S. typhimurium* were equally prevalent in cattle. Thus of 39 isolates, 18 were *S. dublin*, 17 *S. typhimurium*, 3 *S. enteritidis* and 1 *S. bareilly*.

Other reports of *S. dublin* infection came from the Sudan, where Quddus Khan (1962a, b) recorded its isolation from 1 of 161 specimens examined during an abattoir

survey; from Ceylon, where Bandaranayake & Thambiaiyah (1961) described serious outbreaks in calves, and from Mozambique, where Valadão (1961) stated that 11 of 15 isolates from cattle were of this serotype.

In North America, *S. dublin* infection still seems to be confined to the west. Thus when Moran (1962) reviewed 6216 strains of *Salmonella* isolated during a period of $4\frac{1}{2}$ years she found that 185 (42%) of the bovine strains were *S. typhimurium*. Fifty strains were *S. dublin* but all of these came from the west. The other isolates from cattle comprised 33 different serotypes. Similarly, the outbreak in adult cattle reported by Schroeder & Dale (1960) occurred in Los Angeles, California. This outbreak was of interest because, as is often the case, the origin of the investigation was the occurrence of *S. dublin* infection in man. In the east of the U.S.A., however, Mann (1963), who examined the mesenteric lymph nodes of 200 cattle, showed the presence of *S. typhimurium*, *S. derby* and *S. muenchen* but failed to isolate *S. dublin*. Similarly, Ellis (1962) did not encounter *S. dublin* infection among the 40 outbreaks of salmonellosis that he saw in Florida during a 2-year period. Similar findings were reported from Canada by Bigland (1962), who listed the salmonellae isolated from animals and birds in Alberta during the period 1949–60. Twenty-four isolates were from cattle but none of these, and none of the 164 isolates from other host species, was *S. dublin*.

Elsewhere, other authors have recorded the continued absence of *S. dublin*. In South America, Arroyo & Bolaños (1960) reported the examination of apparently healthy cattle slaughtered in Costa Rica. Salmonellae of 8 serotypes were isolated from 26 (13%) of 195 adult cattle. *S. panama* was the commonest serotype. *S. typhimurium* was found, but not *S. dublin*. Nottingham & Urselmann (1961) reported a $2\frac{1}{2}$-year survey made at meat works in New Zealand. Salmonellae were isolated from about 15% of the 133 cows examined, from about 13% of 1110 calves and from 4% of 48 beef cattle. About 10% of the isolates were *S. bovis-morbificans*, and a few were *S. anatum* and *S. newington*. All the remainder were *S. typhimurium*. *S. dublin* was not found although, as mentioned previously, it has been isolated in New Zealand from man (Salisbury, 1958). Le Noc (1963) listed the salmonellae isolated up to 1962 in Madagascar, and noted that *S. typhimurium* was the only serotype obtained from cattle. Similarly, Sakazaki (1961) listed the isolates obtained in Japan between 1949 and 1957. Cattle had yielded *S. nagoya* and *S. enteritidis* but not *S. dublin*.

These references to the incidence of *S. dublin* infection have already given some indication of the incidence of infection with *S. typhimurium* and other salmonellae. It seems unnecessary to list all of the many other references to such isolations from cattle, but it is of interest to note that Asdrubali & Coppini (1961) described *S. typhimurium* as the predominant salmonella infection of cattle in Perugia, Italy, and that important outbreaks with this serotype were reported by Sandbu (1960), Hoflund (1961), Angus & Barr (1963), Gregorovíc Brglez, Klemenc, Skušek & Šenk (1963), Rude (1963) and Schaal (1963). In addition, Cameron, Tustin & Meeser (1963) reported its occurrence in blue wilde-calves (*Connochaetes taurinus* (Burchell)) in the Kruger Park, South Africa, and suggested that it was endemic in the Blue Wildebeest there.

A wide variety of other serotypes was isolated from cattle during the period under review, supporting the view that any salmonella is likely to occur in cattle from time

to time, although probably only as an intercurrent or opportunist infection. Thus at Ghent, Geurden, Devos & van den Wyngaert (1960) isolated *S. berta* from a calf with polyarthritis. Sakazaki (1961) isolated *S. nagoya* from cattle in Japan, and Soeratno (1961) obtained *S. javiana* and *S. weltevreden* from cattle in Indonesia. Investigations in countries not previously surveyed can be expected to produce a variety of unusual or new serotypes. Thus Zwart (1962), who surveyed abattoir cattle in Ghana and found that about 21 % yielded salmonellae, isolated 11 new serotypes. Similarly, Le Minor, Thome, Perreau & Charié-Marsaines (1959) isolated two new serotypes, *S. millesi* and *S. tchad*, from the mesenteric lymph nodes of cattle in Chad.

As discussed earlier, many early references to *S enteritidis* are unreliable because this organism was not differentiated from *S. dublin* until 1930 (White, 1930). It is therefore of interest to note reports of its continued occurrence from various parts of the world including Japan (Sakazaki, 1961; Akasawa & Enomoto, 1963), where it was said to be the commonest serotype in cattle, and from Roumania (Sirmon, Minăscurtă & Badea, 1962).

Similar confusion existed between *S. paratyphi* B, *S. typhimurium* and other serologically related serotypes, and because of this reports of isolations of *S. paratyphi* B from farm animals are usually viewed with caution. However, Burtikashvili (1960, 1961) reported its isolation from buffaloes in Georgia, U.S.S.R., and Röhr (1961) reported that 4 isolations were made at Potsdam from calves and older cattle between 1951 and 1960. Lenk, Rasch & Bulling (1960), who commented that such reports are rare and are received with caution, described the isolation of *S. paratyphi* B from a cow believed to have been infected by a human carrier. They gave details of the differentiation of the isolate from *S. java*, the serotype that most closely resembles *S. paratyphi* B.

Some outbreaks involve more than one serotype. Morten (1962) described an extensive outbreak in a dairy herd in which both *S. typhimurium* and *S. anatum* were present, and Sirmon *et al.* (1962) described one involving *S. typhimurium* and *S. enteritidis*. Sometimes a wider variety of serotypes is found, suggesting that the food or water of the herd is contaminated. Thus Avery & Niilo (1963) reported a herd outbreak in South Alberta, Canada, involving 5 serotypes. Examination of an unopened sack of bonemeal yielded 3 of these together with 2 other serotypes.

Other aetiological factors

Although this review deals at length with the various serotypes isolated from cattle, it would be wrong to leave the impression that the causal organism is the only aetiological factor worthy of consideration. The literature abounds in references to the importance of various physiological, nutritional and management factors, and the presence of intercurrent infections, in either rendering cattle more susceptible to infection or in activating what would otherwise remain a latent infection, and thus producing either the active carrier state or clinical disease. Indeed, this is a fundamental aspect of the epidemiology of salmonella infections in any host species (Lovell, 1940, 1953; Buxton, 1957*b*).

Thus, in considering the causation of salmonellosis in calves in Brazil, Dupont (1932) commented that it chiefly affected those that were anaemic because of piro-

plasmosis and anaplasmosis. Piening (1938) reported that both in calves and in adult cattle, the incidence of salmonella infection was higher in those slaughtered because of foot-and-mouth disease than in uninfected cattle. Guerrero (1943) described various secondary factors, both of management and disease, that resulted in a high incidence of salmonellosis in the warm rainy season in Columbia and Ecuador. Köbe & Heinig (1939) offered similar observations from the Rheims Foot-and-Mouth Disease Institute. Salmonellae, chiefly *S. typhimurium*, were isolated from the faeces of 1·5 % of cattle entering the institute, but the incidence rose to 4·7 % after virus infection and to about 10 % in those dying from foot-and-mouth disease.

Hutyra, Marek & Manninger (1938) reviewed the epidemiology of *S. dublin* and *S. typhimurium* infection in adult cattle. They stated that some clinical cases, referred to as 'secondary paratyphoid', arose when the resistance of 'carrier' animals was reduced by intercurrent disease. Other cases ('primary paratyphoid') were attributed to unfavourable external conditions such as poor feeding and the effects of transportation. Olson (1939), Clarenburg & Vink (1949) and Field (1949 a) all attached importance to similar secondary factors. Gibson (1958) provided further examples of their importance and attributed the seasonal incidence of the disease in adult cattle in south Wales to a combination of factors of climate and intercurrent disease.

Further evidence has accumulated during the period under review, both with regard to bovine salmonellosis and to the disease in other species. Indeed, it would seem that the importance of such 'stress factors' is now so widely accepted that they are sometimes invoked on very slender evidence. The more convincing reports include one from Bechuanaland (Report, 1960) attributing the very heavy calf losses from salmonellosis to the husbandry system and the very poor hygienic conditions under which calves are kept. It commented that although extensive use was made of a vaccine it was inadequate in the face of these conditions. Buxton (1960) described the secondary factors affecting calves under British conditions, and emphasized the harmful effect of long journeys, poor housing and dietary abuse, points that were also emphasized by Gibson (1961) and Preston (1963). Ellis (1962), Moore, Rothenbacher, Bennett & Barner (1962) and Avery & Niilo (1963) also stressed the importance of various secondary factors in the outbreaks that they reported in calves and older cattle.

It has been recognized for some time that the conditions under which animals are transported and under which they are held in lairages awaiting slaughter are conducive to cross-infection with salmonellae. Anderson *et al.* (1961) and Galbraith (1961) reported evidence of such cross-infection in calves. Thus, in one investigation, salmonellae were isolated from only 1 of 156 calves slaughtered within a few hours of arrival at the lairage, but from 59 (44 %) of 133 kept there for several days. In adult cattle, Morten (1962) confirmed that salmonellosis tends to be more severe in newly calved cows, Zagaevskii (1962) confirmed the importance of concurrent fascioliasis as a secondary factor, and Uziębło (1961) provided further evidence of the association between rough sour pasture and endemic salmonella infection.

In summary, the current literature confirms that *S. dublin* remains an important specific infection of cattle; that *S. typhimurium* infection is also important and widespread, and that a wide variety of other serotypes are found from time to time,

especially in cattle exposed to contaminated food, water or environment. It also provides further evidence of the great importance of secondary factors in the aetiology of salmonella infections in cattle.

PATHOGENESIS

A detailed consideration of the pathogenesis of salmonellosis is outside the scope of this review, especially as little of the experimental work concerned has been done in cattle. It should be mentioned in passing, however, that salmonellae are commonly used to study the basic mechanisms of infection and resistance in experimental animals.

A point deserving consideration is the route of infection. It is generally stated that infection is usually by mouth (Buxton, 1957b), but Moore (1957) showed that the conjunctiva could also be an important point of entry. Pritulin (1959) used face masks to infect sheep and calves with salmonella cultures in aerosol form. He reported that the minimum infective dose was 3 or 4 times lower than when the organisms were given orally and later (Pritulin, 1961) advocated that this technique should be used for immunization. Darlow, Bale & Carter (1961) infected mice by the respiratory route and also found the infective dose to be much smaller than that for oral dosage.

In the reviewer's experience the dissemination of contamination within an infected building is much wider than the visible soiling with faecal material would suggest, indicating that the animals in such a building have been exposed to aerosols of the organism. This suggests that further studies on the respiratory and conjunctival routes of infection might well prove rewarding.

In adult cattle, infection may be followed by clinical disease, by the active carrier state, or by latent carriage of the organism (Gibson, 1958; Field, 1959). Although the clinical disease is usually sporadic, it is not uncommon for 2 or 3 cases to occur in a herd at intervals of 2–3 weeks. This suggests that the incubation period is usually of this duration. As indicated on p. 104, the latent carrier state may be activated by intercurrent disease or by adverse environmental factors to give rise either to the active carrier state or to clinical disease, perhaps years after the initial infection.

EFFECTS OF SALMONELLA INFECTIONS IN CATTLE
Clinical manifestations

The variable clinical picture shown by infected calves is briefly described in the introduction to this review. It is well recorded in the earlier references (for example by Field, 1948, 1959) and the period under review has added little to this subject.

The clinical disease in older cattle was also well documented before 1960, but one feature that deserves special mention is the occurrence of abortion. It was already established that pregnant cows that develop clinical salmonellosis commonly abort (Olson, 1939; John, 1946), even if the clinical signs of the disease are less severe than usual (Field, 1948), and that the calves of active carriers may also be aborted and yield the organism on cultural examination (Bert, 1943; Field, 1948; Gibson, 1958). Turning to the more recent references, abortion was a feature of the outbreaks recorded by Avery & Niilo (1963) and by Bulling (1961). Van Ulsen (1960) reported that *S. dublin* was isolated from 113 (1·2 %) of 9723 bovine foetuses, aborted after

3–9 months gestation, and examined in Holland between 1950 and 1959. The incidence of *S. dublin* infection rose during the survey period from 0–0·7 to 1·5 %, and showed a peak seasonal incidence in January and August. Liebermann *et al.* (1960) reported the isolation of *S. dublin* from two (1·1 %) of 189 foetuses examined in Poland during 1959. Apart from frank abortion, salmonella infection may also be associated with stillbirths and neonatal deaths (Field, 1948; Geurden, Devos & De Vos, 1949).

Excretion of the causal organism

It is well recognized that adult cattle that recover from clinical infection with *S. dublin* usually continue to excrete the organism in their faeces for many years if not for life (Proescholdt, 1931; Field, 1948; Gibson, 1958). This constant excretion may also be shown by cattle with no history of clinical disease (Field, 1948; Gibson, 1958). In other cattle excretion of the organism may be intermittent (Rievel, 1933; Trawinski, 1957; Gibson, 1958). Excretion may also occur in the urine (Trawinski, 1957). Infection with *S. typhimurium* may also give rise to the active carrier state, but this seems to be of shorter duration than that produced by *S. dublin* (Report, 1959).

Little further evidence on these points was published during the period under review. However, Morten (1962) recorded an outbreak in which *S. typhimurium* was isolated from the faeces of 83 members of a dairy herd. Six were still positive 6 months later and 1 at 10 months.

Another point of interest is the length of time for which calves continue to excrete the causal organism. Field (1948) stated that, as judged by the examination of faeces, calves do not remain carriers of salmonellae. It seems, however, that exceptionally a calf may remain an active carrier into adult life (Field, 1959). An interesting observation was published by Sandbu (1960), who examined 23 calves that had survived an outbreak of *S. typhimurium* infection. Their faeces were negative but the organism was isolated from the viscera of 17, especially from the mesenteric lymph nodes and liver. It is self-evident that this is of considerable public health significance.

Gibson (1958, 1961) emphasized that in calves excretion of the organism tends to be intermittent.

Stellmacher (1963a) reported that salmonellae could be recovered from the faeces of calves 1 day after they had received a massive oral dose of organisms, or 2 days after a moderate dose. No clinical signs were caused and excretion ceased when dosing was discontinued. Avery & Niilo (1963) reported similar findings from dosing 1 adult cow with contaminated bone meal.

Another point in adult cattle which is of considerable public health significance, is that of the likelihood of the excretion of salmonellae in the milk of dairy cows. It is generally agreed that such excretion is commonly shown during the febrile stage of clinical salmonellosis. This has been reported for *S. dublin* infection (Tulloch, 1939; Field, 1948; Grunsell & Osborne, 1948; Gibson, 1958), *S. thompson* (Wright, Norval & Orr, 1957) and for *S. newport* (Boyd, 1958). The earlier reports are equivocal as to whether active carriers excrete the organism in their milk. Thus Rievel (1933) failed to isolate the organism from the milk of 4 active carriers of '*S. enteritidis*'. Standfuss,

Wilken & Sörrensen (1932) made occasional isolations from the milk of 6 cows but stated that these could have been due to faecal contamination, and it is, of course, precisely this possibility that makes it difficult to assess the many claims in the literature. This was shown by Harms (1959) when he examined 2 active carriers of *S. dublin*. He found that 12 samples taken with teat siphons were negative, but that when hand-milking was used, without first washing the udder, 14 (36 %) of 39 samples yielded the organism. Gibson (1958) examined 71 samples from various active carriers of *S. dublin* and made only one isolation.

Some of the evidence presented during the period under review is equally equivocal. An interesting exception, however, was provided by Davies & Venn (1962) who isolated *S. heidelberg* from one quarter of the udder of a cow, both during life and post mortem. This cow was the probable source of an explosive outbreak of food poisoning in the human population.

It is also of interest to note that Arkhangel'skii & Kartashova (1962) reported on the use of fluorescent microscopy as a rapid method for the detection of salmonellae in milk. Using artificially contaminated milk they found that the fluorescent method was reliable for samples containing 100000 organisms/ml, but that cultural examination was necessary to detect smaller numbers, such as 100/ml.

Serological response to infection

The range of agglutinins to salmonellae shown by normal and infected cattle has been discussed by various workers including Lütje (1940), Field (1948), Clarenburg, Vink & Schuurmans (1950) and Gibson (1958). Allowances must be made for the different techniques used, but generally speaking it is found that normal adult cattle seldom show somatic ('O') agglutinins above a titre of 1:40 or flagellar ('H') agglutinins above 1:160. Field (1948) showed also that young calves exhibit little or no production of somatic agglutinins in response to infection. This was confirmed by Gibson (1958, 1961).

During the period under review Malyavin & Gol'denman (1961) reported on the transfer of agglutinins from hyperimmunized cows to their calves via the colostrum. They demonstrated agglutinins in the blood serum of the calves within 2 h of their taking colostrum. Another Russian study of the blood titres of cattle was by Zagaevskii (1962).

Pathological changes

The gross pathology of bovine salmonellosis is well described in the standard texts. One point of interest is that reports from the mainland of Europe commonly refer to the presence of necrotic foci in the liver and kidneys of calves dying from sub-acute salmonellosis (Langer, 1904; von Hutyra *et al.* 1938). Stoll (1960) reported on the bacteriological examination of 103 calves showing this type of miliary necrosis of the liver; bacteria were isolated from 99 of them. Forty-one of the isolates were *S. dublin* but no other salmonellae were found. Gibson (1961) commented that lesions of this kind are not commonly found in cases occurring in Britain.

Borodulina (1960) studied the histopathology of the brain of young calves infected with various organisms including salmonellae.

INCIDENCE OF BOVINE SALMONELLOSIS

Geographical incidence

This was discussed at some length on pp. 100–104.

General incidence and economic importance

It has been indicated on p. 102 that bovine salmonellosis is not only widespread but can also be of economic importance. In Britain the disease appears to be of increasing importance in calves. This view is supported by the incidence of salmonella infection in calves diagnosed at Veterinary Investigation Centres in England and Wales during the 3 years of 1958–60 (Gibson, 1961). The yearly totals were 123, 369 and 425, respectively. Preston (1963) described salmonellosis as the most important disease facing the calf rearer. It is difficult to assess the present significance of bovine salmonellosis in other countries. It would seem, however, that the pattern shown in Britain is likely to be repeated elsewhere if similar methods of intensive husbandry are introduced.

Seasonal incidence

In adult cattle, salmonellosis occurs more commonly in summer and autumn, when they are at pasture. Field (1948) found that 57 (84 %) of 68 outbreaks in south Wales in 1946–47 occurred during the period of June–October inclusive and in a smaller series of cases in the same area Gibson (1958) found that 13 (93 %) out of 14 commenced during the same period. Grunsell & Osborne (1948) found a similar seasonal incidence in Somerset, as did Olson (1939) in Sweden and Clarenburg & Vink (1949) in Holland. More recently, Bulling (1961) reported the same seasonal incidence (i.e. June–October) for salmonellosis due to *S. dublin* infection in adult cattle in Lower Saxony. The seasonal incidence of *S. typhimurium* infection in adult cattle seems to be similar. Vasenius (1959) reported a series of cases occurring between July and August. Rude (1963) found that 8 of 9 cases occurred in October.

In calves the seasonal incidence is variable, depending both on the calving season and on the incidence of any of the various secondary factors that can precipitate or aggravate the clinical disease. Guerrero (1943) reported that in Columbia and Ecuador salmonellosis was chiefly a disease of the hot rainy season. Lerena (1946) also associated the disease with the hot season in Brazil. Gibson (1961) reported that both fatal and mild cases were more common in Britain in autumn and winter, and associated this with the numbers at risk at that time and the adverse environmental factors to which they were exposed. Nottingham & Urselmann (1961) noted spring and autumn peaks in the incidence of infection found in an abattoir survey in New Zealand. Rude (1963) found that 31 (84 %) of 37 outbreaks of *S. typhimurium* infection diagnosed in Wisconsin, U.S.A., occurred in September–March inclusive.

Age incidence

Gibson (1961) gave data indicating that in calves salmonellosis is more prevalent after 1 week of age than before. There appears to be no age incidence for the disease in older cattle.

EPIDEMIOLOGICAL CONSIDERATIONS

Introduction of infection

Theoretically, infection could be introduced to a herd by any one of a number of routes; by the purchase of infected animals, by contamination of food, water or fertilizers, by cross infection from other domestic animals, or from man, or from various free-living animals, birds, or other forms of animal life. The literature contains many records in which these various factors are suggested as the possible or probable origin of infection. Sometimes, however, there is little or no supporting evidence for the suggestions. In considering such possibilities it should be remembered that *S. dublin* shows a considerable degree of host specificity. It occasionally causes outbreaks of abortion in sheep (Shearer, 1957; Watson, 1960) and, as in Scandinavia (Wramby, 1937; Momberg-Jørgensen, 1949), can be an important cause of losses in silver foxes and other fur-bearing animals. Such incidents, however, are uncommon compared with its occurrence in cattle. Thus Gibson (1961) stated that 914 (94%) of 969 strains of *S. dublin* isolated at Veterinary Investigation Centres in England and Wales during the 3 years of 1958–60 were from cattle. It will be appreciated that because of this degree of host specificity the most likely source of *S. dublin* infection in cattle is contact, direct or indirect, with other infected cattle.

A few other salmonellae show a similar host specificity, notably *S. pullorum* and *S. gallinarum* for poultry, and *S. cholerae-suis* for pigs. They may, nevertheless, occasionally be found in cattle (Buxton, 1957b), undoubtedly as the result of direct or indirect contact with the specific host. Thus the outbreak of *S. cholerae-suis* infection in cattle reported by Hoflund (1961) was associated with pollution of pasture with water from a piggery. In contrast to this, *S. typhimurium* and most of the other salmonellae show a complete lack of host specificity, and bovine infection could therefore originate from any of a very wide range of host species.

It will be convenient to consider the various possible sources of infection separately, but before doing so it should be re-emphasized that a considerable number of clinical cases in adult cattle are probably of endogenous rather than exogenous origin in that they represent an activation of a latent infection (Gibson, 1958; Field, 1959), and that the introduction of infection may sometimes precede the development of clinical disease by several months or years. Failure to recognize this has led some investigators to attribute infection to somewhat unlikely sources.

Introduction by purchase

It is obvious that clean premises may become contaminated if infected calves or older cattle are purchased. This is of great importance when calves are collected from various sources for intensive rearing. Gibson (1961) presented evidence that both clinical and subclinical infection of calves can arise in this way. Infection introduced by purchased animals may have been acquired on their home premises or in transit, in markets, in collecting centres or in lorries. Jørgensen (1962) demonstrated the latter hazard when he isolated salmonellae from water that had been used for washing soiled cattle lorries, and, as previously noted, Anderson *et al.* (1961) demonstrated the build up of infection that can occur among calves in collecting centres.

Nottingham & Urselmann (1961) also noted that calves that had been a shorter time in transit and in the lairage showed a lower incidence of salmonella infection.

Introduction with water and drainage

It is notoriously difficult to trace the origin of an existing outbreak of bovine salmonellosis, and much evidence cited as incriminating water supplies or drainage is therefore circumstantial. However, Steiniger (1954) attributed an outbreak in cattle close to the Elbe estuary to flooding of pasture with contaminated sea water. Bederke & Lundt (1954) attributed bovine infection to the contamination of water meadows with human sewage, as did Holz (1956) and Strauch & Münker (1956). It is noteworthy, however, that although the latter authors regarded the contaminated river water as a likely source of the bovine infection, the strains isolated from it did not correspond to those from the cattle. Various other workers have demonstrated the presence of salmonellae in river water, especially downstream from the outfalls of town effluents or abattoir wastes (Ljutov, 1954; Phiening, 1954; Pohl, 1955; Rasch, 1955). Rasch & Richter (1956) isolated *S. heidelberg* from a stream after liquid manure had been spread on an adjacent field and believed that this was the origin of infection in cattle grazing a pasture downstream. Gibson (1958) isolated *S. dublin* downstream from infected premises in south Wales. Details of suitable techniques for such work were given in a report (Report, 1959a) on the contamination of British coastal waters.

Similar observations have been reported during the period under review. Fey & Vallette (1961) isolated salmonellae of 18 serotypes from 5 of 18 samples of abattoir effluent, from 18 of 32 samples taken from rivers at sewage outfalls, and from 7 of 21 other samples of river water. Van der Schaaf & Hagens (1963) also isolated salmonellae from sewage effluent and from sludge. Schaal (1961) showed, moreover, that under favourable conditions *S. dublin* can multiply in waste water at 18–20 °C. In a later paper Schaal (1963) attributed an outbreak of *S. typhimurium* infection in a large herd to heavy contamination of the brook from which they drank. The outbreak was brought under control by measures that included fencing this brook and providing drinking troughs. Hoflund (1961) attributed *S. cholerae-suis* infection in cattle to pollution of pasture by water from a piggery, and *S. typhimurium* infection in another herd to pollution with water from a housing estate. Jørgensen (1962) isolated salmonellae from 'purified' sewage effluent and from a stream contaminated with abattoir waste and sewage. He failed, however, to demonstrate infection in calves and yearling cattle that grazed pastures irrigated weekly with water from this stream.

Introduction in feedingstuffs and fertilizers

The fact that many consignments of fish meal, bone meal, meat and bone meal and similar organic materials contain a variety of salmonellae has attracted much interest during the past 12 years. Such products are, of course, widely used in feedingstuffs for adult cattle and as ingredients of 'milk substitutes' and other calf foods. Foods intended for young calves are of special interest because, as in other species, these animals constitute the most susceptible age group (Edwards *et al.* 1948; Buxton, 1957b). Before considering ingredients of this kind, however, it is of interest to note

that simple milk products can also be a source of infection to calves. Grini (1949) suggested that an outbreak of *S. dublin* infection in west Norway was possibly due to contamination of skim-milk returned from the creameries, and Simmons & Sutherland (1950) suspected whey from a cheese factory to be the vehicle of *S. typhimurium* infection in Queensland, Australia. More recently, Sirmon *et al.* (1962) incriminated milk in an outbreak in Roumania. The previous discussion of the excretion of salmonellae in milk on p. 107 is obviously relevant here.

Investigations into the salmonella content of feedingstuffs and fertilizers have resulted in the isolation of a wide range of serotypes. In Britain (Report, 1959b) it was found that salmonellae were present in many consignments of the raw materials and also in the finished products. Altogether, 88 serotypes, including 6 new ones, were found, the highest incidence being in imported bones and bone products and in Angola fish meal. Similarly, in Sweden, Rutqvist & Thal (1958) isolated 161 strains of 32 serotypes from imported meatmeal and, in Denmark, Müller (1959) found 29 serotypes, including many not previously recognized in that country. Hauge & Bøvre (1958) found salmonellae in feedingstuffs of vegetable origin, with a high incidence (16 % of 200 samples) in Mexican cotton-seed cake. Müller (1959) and Jensen (1959) also made isolations from foods of vegetable origin.

In addition to showing the presence of salmonellae in these products, several workers published circumstantial evidence that contaminated foods could produce clinical disease or the carrier state. Müller (1959) reported on the necropsy of calves in Denmark and noted an increasing incidence of serotypes not previously found in that country. He associated this with the importation of meat and bone meal and of fish meal, some samples of which yielded the same serotypes. He noted a similar increase in 'exotic' salmonellae in poultry and in man during the same period. Gray *et al.* (1958) attributed an outbreak of salmonellosis involving 9 dairy farms in Victoria, Australia, to the use of bonemeal that contained a variety of salmonellae. Newell, McClarin, Murdock, MacDonald & Hutchinson (1959) offered similar evidence linking infection in pigs and in pig food in Northern Ireland.

During the period under review many additional studies have been published, and these have amply confirmed that feedingstuffs of animal origin commonly contain salmonellae. Thus Röhr (1960) reported the isolation of 1390 strains of 66 serotypes from 1200 (23·6 %) of 5066 samples examined at Potsdam during a 6-year period. *S. cubana* and *S. derby* were the commonest serotypes. In Belgium, Thomas (1961) reported that 92 (5·6 %) of 1638 samples of meat meal and fish meal, mainly imported, yielded 125 strains of 42 serotypes. Kampelmacher *et al.* (1962) reported the isolation of 573 strains of 79 serotypes in Holland during the period 1955–60, and noted that both the number of isolations and the number of serotypes showed a steady increase during this period. Wedman (1962) reported that correlation of previous American findings showed that 718 (13 %) of 5712 samples of animal byproducts and feedingstuffs had yielded salmonellae of 62 serotypes. Gray, Harley & Noble (1960) stated that 91 % of Australian home-produced bone meal was positive for salmonellae. Stenberg (1963) found salmonellae in 19 % of 240 samples of Finnish home-produced bone meal and meat meal and other feedingstuffs. The most spectacular finding of all, however, was that of Harvey & Price (1962) who reported that 56 of 57 samples of Indian crushed bone yielded salmonellae and that up to 17 serotypes were isolated

from each sample. Salmonellae may also be found in fertilizers containing bone meal. However, Dixon & Wilson (1960) reported that the organisms showed poor viability when contaminated bone meal was mixed with superphosphate as in normal commercial fertilizer practice. They concluded that there was no reason to believe that these compound fertilizers were of importance in the epidemiology of salmonella infection in animals.

Other workers have confirmed that foods of vegetable origin may also be contaminated. Thus in a survey in Britain (Report, 1961) examination of raw ingredients of vegetable origin used by one feedingstuffs company showed that 3 (1·7%) of 175 samples contained salmonellae, while 5 (6·5%) of 77 samples taken from various other companies were also positive. Similar findings have been reported by various workers including Grumbles & Flowers (1961), Rutqvist (1961), Huisman & Daniëls-Bosman (1961) and Rutqvist & Waxberg (1963).

It is obviously necessary to keep the subject of food contamination in perspective. First, it is important to consider the degree of contamination in the final product, as fed to the animal, rather than that in the raw ingredients. Thus the British survey of the problem (Report, 1961) showed that although examination of the raw ingredients used by one company disclosed an incidence of 9%, with some ingredients yielding from 11 to 19% of positive isolations, salmonellae were present in only 2·8% of finished meals and in only 0·27% of pelleted foods. Moreover, the numbers of salmonella organisms present in the raw ingredients were small—less than 30/100 g— and were usually reduced by 80–98% by the heating associated with the process of pelleting. It was concluded that on the few occasions when salmonellae were present in pelleted foods their numbers were likely to be very small and that, in one series of examinations, pelleted foods could, for practical purposes, be regarded as free from salmonellae.

Secondly, the salmonellae isolated from feedingstuffs seldom include *S. typhimurium* and only very rarely *S. dublin*—the 2 serotypes which are chiefly associated with clinical salmonellosis in cattle. Thus in the British survey (Report, 1961) one series of examinations showed that only 5 (2·4%) of 209 isolates were *S. typhimurium*. None was *S. dublin*. In a second series 3 (8·1%) were *S. typhimurium* and none *S. dublin*. Thomas (1961) reported that 5 (4%) of 125 strains isolated from foods in Belgium were *S. typhimurium*. None was *S. dublin*. In Holland, Kampelmacher *et al.* (1962) found that only 22 (3·8%) of 573 strains were *S. typhimurium*, and only 2 (0·3%) *S. dublin*. Wedman (1962) stated that of 718 isolates made in the U.S.A., 27 (4%) were *S. typhimurium*. None was *S. dublin*, a finding in keeping with the apparent absence of *S. dublin* from the greater part of the U.S.A. (see p. 103). Pomeroy & Grady (1962) reported similar results. Harvey & Price (1962) stated that only 6 (3%) of the 226 strains isolated from 57 samples of Indian bone meal were *S. typhimurium*. One was *S. dublin*.

A limited number of feeding experiments have been conducted in an effort to elucidate the significance of the contamination of feedingstuffs. Important evidence was presented by Smith (1960), who worked with pigs, 20 of which were fed a normal-type ration containing 10% of Angola fish meal and 2% of Pakistan bone meal, both known to be heavily contaminated with various salmonellae. None of the pigs showed any ill-health. Salmonellae were found in very small numbers in the mesenteric

lymph nodes of some, but not in other internal organs or in the muscular tissue. The faeces were found to contain these organisms from time to time, but none of the pigs became a permanent excretor of salmonellae. A noteworthy point is that *S. typhimurium* was the most common serotype found in the mesenteric lymph nodes although it was probably present in the food in much smaller numbers than the other serotypes. This suggests a selective action of considerable importance from the public health aspect.

Smith also reported a small experiment in which two 4-month-old calves were fed a bone meal containing both *S. typhimurium* and *S. dublin*. They remained healthy and neither of these 2 salmonellae was isolated from their faeces in life or from their organs post-mortem. Avery & Niilo (1963) reported that a single cow dosed with naturally contaminated bone meal excreted the organism in her faeces but showed no clinical signs and was culturally negative when examined after slaughter. One is left with circumstantial evidence that contaminated feedstuffs may act as the source of some salmonella infections in cattle, but it appears that *S. dublin* infection seldom if ever arises in this way because of the rarity of this serotype in feedingstuffs. *S. typhimurium* is less rare and, as indicated above (Smith, 1960), may act selectively. Even so, it seems unlikely that many infections arise in this way. The evidence is more convincing when the less common serotypes are considered, especially ones previously rare or unrecorded in a given country. Thus Galbraith, Archer & Tee (1961) considered that the incidents of *S. saint-paul* infection that occurred in Britain in 1959 probably originated in this way. Knox, Galbraith, Lewis, Hickie & Johnston (1963) attributed infection of a dairy cow with *S. heidelberg* to contaminated feeding-stuffs and were able to demonstrate the presence of the organism in the mill that supplied the affected farm. Conversely, Röhr (1962) reported that infection of cattle with *S. taksony* disappeared spontaneously when contaminated food was withdrawn. Supporting evidence is also available from other animal species. Thus Boyer, Narotsky, Bruner & Brown (1962) reported a general correspondence between isolates from poultry and from feedingstuffs. They also reported 3 outbreaks in which the same organism was isolated from birds and from unopened bags of food.

These 'exotic' serotypes rarely cause clinical disease in cattle, and it seems that the chief significance of their presence in feedingstuffs is that they may occasionally cause subclinical or latent infections in cattle which could then act as the source of human infection.

Introduction by other domestic animals or by man

As stated on p. 110, this route is less likely to be implicated in cases of *S. dublin* infection because this serotype shows a considerable degree of host specificity for cattle, with the result that most outbreaks in cattle are due to contact, direct or indirect, with other infected cattle. Domestic animals and man could, of course, provide this indirect contact by mechanical transportation of the organism.

True infection with *S. dublin* has, however, been reported by various authors in a wide range of domestic animals including sheep (Nordlund, 1938; Shearer, 1957), goats (Levi, 1949; Gibson, 1957), pigs (Lütje, 1938–39; Gordon, 1951; McErlean, 1956), fowls (Lütje, 1937; Hansen, 1942; Nordberg & Ekstam, 1950), pigeons (van Dorssen, 1936), horses (Knoth, 1936; Smith & Buxton, 1951; Hirsch, 1952; Mullaney,

1955), dogs (Rislakki & Stenberg, 1953) and animals kept for fur (Wramby, 1937; Momberg-Jørgensen, 1949). The first strains of *S. dublin* to be differentiated from *S. enteritidis* were isolated from man (White, 1930) and many other isolations have been recorded since then (Tulloch, 1939; Sutherland & Berger, 1944; Cromb & Murdock, 1949). Bulling (1961) confirmed this wide host range of *S. dublin*. He also isolated this serotype from geese, as did Watson (1960) and Zagaevskii (1961). Various workers have reported further isolations of *S. dublin* from the host species listed above, including isolations from sheep (Watson, 1960), pigs (Valadão, 1962; Kampelmacher *et al.* 1962; Geurden, Devos, Viaene & Staelens, 1963), fowls (Rao & Gupta, 1961), pigeons (Schulte & Scholz, 1960; Thomas, 1961) and dogs (Watson, 1960; Gibson, 1961; van der Schaaf, 1961).

It will be seen that theoretically there is a wide range of possible sources of *S. dublin* infection among domestic animals. However, it must be emphasized that its occurrence in these species is rare compared with its incidence in cattle. Moreover, many of the references cited above refer to the latent carrier state and not to clinical disease or active excretion of the organism. It therefore appears that except under special circumstances—as when an outbreak of *S. dublin* abortion in sheep causes heavy contamination of the pasture—infection in other domestic animals or in man presents little hazard to cattle compared with the grave risk deriving from bovine active carriers.

As stated above, the host-specific serotypes, such as *S. pullorum*, *S. gallinarum* and *S. cholerae-suis* are very occasionally isolated from cattle. The introduction of poultry or pigs infected with these serotypes might therefore constitute a hazard to cattle, but it must be emphasized that the risk of this kind of cross-infection is slight.

When infection with *S. typhimurium* and the other non-specific serotypes is considered the possible role of other host species becomes more important. Stenert (1938) isolated *S. typhimurium* from geese, ducks and a bull on one farm, and Sellers & Sinclair (1953) from cattle, pigs, fowls and ducks on another. In the latter outbreak all the isolates were of the same phage type, indicating an epidemiological relationship. Müller (1957) stated that in Denmark *S. typhimurium* infection in calves is often traced to poultry. Klotz (1959) isolated *S. typhimurium* from ducks and calves and attributed an extensive outbreak in the latter to the addition of duck eggs to their milk. Clarenburg & Vink (1948) attributed *S. anatum* infection in cattle to infected ducks found on the same farm. Similar observations have been recorded during the period under review. Nielsen (1963) reported the isolation of *S. typhimurium* from a dead calf and from the faeces of 51 fowls. The isolates were of the same phage type. Messerli (1962) recorded an outbreak in which a farmer's wife, the farmer and three young calves were ill, in that order, with *S. typhimurium* infection, and considered the bovine infection to be secondary to the human one.

Although other domestic animals and man must be considered as a possible source of bovine infection with the non-specific serotypes, the possible role of other cattle must not be overlooked. This is especially true of *S. typhimurium*, which commonly causes a continuing infection within a herd.

Introduction by free-living animals and birds

Rats are commonly described as a likely source of salmonella infection, and especially of infection with *S. typhimurium*, in domestic animals and man. Their role in *S. dublin* infection appears to be limited. Nordlund (1938) attributed infection of calves and lambs to rats, and demonstrated *S. dublin* in rats caught on the affected premises. Field (1948) also found *S. dublin* in rats caught on or near farms with infected cattle. Gibson (1958, 1961) repeated this observation and found that whereas the incidence of *S. dublin* infection in farm rats examined in south Wales was 8 % on farms with infected cattle, it was only 0·6 % on farms where calves or older cattle excreting *S. dublin* were not known to have been present during the previous 6 months. He suggested that rats might carry salmonellae from infected to uninfected premises, and that they could possibly infect calves by contamination of food, bedding or utensils.

The role of rats as a source of *S. typhimurium* and other serotypes is somewhat controversial. Many workers have regarded rats and mice as a source of *S. typhimurium* infection, both for animals (Lerche & Bartel, 1936; Nordlund, 1938) and for man (Salthe & Krumwiede, 1924; Jones & Wright, 1936). It is true that various workers including Khalil (1938), Ludlam (1954) and Brown & Parker (1957) have isolated *S. typhimurium* and other serotypes from town rats, but less information is available about farm rats. Gibson (1958, 1961) stated that only one of 678 rats obtained from 64 farms in Carmarthenshire, south Wales, yielded *S. typhimurium*, and one other *S. thompson*. Both serotypes were virtually absent from the cattle on the farms from which the rats were obtained, and he concluded that farm rats played little part in the epidemiology of *S. typhimurium* infection in that they did not normally carry this serotype. However, Gregorovíc *et al.* (1963) regarded rats as the source of *S. typhimurium* infection in a herd of dairy calves, as did Zagaevskii (1961) in respect of poultry, and Guinée, Kampelmacher, van Keulen & Ophof (1963) found salmonellae in 17 (4 %) of 429 farm rats. The role of rats may vary from country to country and from one area to another according to the environmental infection to which they are exposed, but it seems that they cannot automatically be regarded as a potential source of salmonella infection. They could, of course, act as mechanical carriers of salmonellae, but the amount of infection transported in this way would seem to be extremely small compared with that excreted by bovine active carriers.

The role of wild birds would seem to be subject to similar limitations. The literature suggests that they play little or no part in the dissemination of *S. dublin* infection, but that they may be associated with *S. typhimurium*. Watts & Wall (1952) found *S. typhimurium* in various carnivorous wild birds when losses were occurring in sheep in south Australia, and Josland (1953) made similar observations in connection with bovine salmonellosis in New Zealand. Fain (1953) regarded rooks as an important source of human salmonellosis in the Belgian Congo. Steiniger (1954) reported that seagulls formed part of an epidemiological complex involving cattle, abattoir waste, man and rodents. Ellemann (1959) found salmonellae in 16 (5 %) of 307 gulls shot near refuse plants and dumps in Copenhagen. Twelve of the 16 isolates were *S. typhimurium*. Hudson & Tudor (1957) reported outbreaks of *S. typhimurium* infection in wild birds in New Jersey, U.S.A. More recent investigations have confirmed the

occurrence of *S. typhimurium* and other serotypes in gulls (Nielsen, 1960; Schwerin, 1960; Sěrý & Strauss, 1960; van Dorssen, van Vloten, Poelma & Zwart, 1960). Mikhaĭlova & Gusev (1960) found salmonellae in wild birds in Dagestan; Dózsa (1961) isolated *S. typhimurium* from 52 (20%) of 266 sparrows originating from the country districts around Budapest; Lofton, Morrison & Leiby (1962) found salmonellae in wild birds in Colorado, U.S.A., and Bigland (1962) in Alberta, Canada.

Various other forms of wild life have been regarded as potential sources of salmonella infection. McAnulty (1958) studied slaughter cattle in Uganda and suggested that some of the serotypes found originated from reptiles or wild game. Goetz (1962) ascribed outbreaks in turkeys to infected wild life.

There are numerous references to the possibility of insects acting as vectors of salmonellae. Trawiński & Trawińska (1960) showed that various serotypes, including *S. dublin*, could be passed from one generation of flies (*Musca domestica*) to the next. Sylwester (1961) offered similar observations. Greenberg, Varela, Bornstein & Hernandez (1963) isolated various serotypes from flies in a slaughterhouse in Mexico. Le Noc (1963) also isolated salmonellae, including *S. typhimurium*, from flies. These findings are undoubtedly of public health significance, but their importance in the spread of salmonella infection among cattle appears problematical.

These references confirm that in some environments wild life could act as a source of salmonella infection for cattle. The risk of such infection would seem to be greater for calves than for adults, partly because of their greater susceptibility and partly because calves have little opportunity to avoid any contamination that gains access to their food. Moreover, under poor hygienic conditions, small numbers of salmonellae gaining access to calf gruels or the utensils used for feeding might be able to multiply to form an infective dose.

In summary, the purchase of infected cattle constitutes a grave danger, especially in calf-rearing establishments. Other possible sources of infection are less easy to assess. It seems that salmonellae are commonly isolated from streams and rivers, but that it is rarely possible to confirm that cattle have acquired infection in this way. Feedingstuffs seem to play little or no part in the epidemiology of *S. dublin* infection and only a minor part in that of *S. typhimurium*. They undoubtedly act as a source of other serotypes, but although these are of public health significance they rarely cause clinical disease in cattle.

An important aspect of the epidemiology of *S. dublin* infection is that in a herd containing one or more active excretors of the organism, it may be several years before further active carriers or clinical cases arise among the remainder of the adult herd. Calves are more likely to acquire infection but, again, this is by no means a constant feature. Given this slow spread of the infection among cattle, it is difficult to attach great significance to the occasional presence of the infection in other domestic animals or in free-living animals and birds, especially if the infection is latent or transient. Further work is required to assess whether this argument is equally valid for infection with *S. typhimurium* and other serotypes, but the reviewer suggests that a comparison between the amount of contamination voided by infected cattle and by other host species will help to keep this subject in perspective.

Dissemination of infection within a herd

This requires little additional discussion. Infection may derive from clinically affected animals or from active carriers showing either constant or intermittent faecal excretion of the organism. It was stated on p. 106 that salmonella infection can cause abortion, stillbirth or neonatal death, and it will be appreciated that the genital discharges from these events are also a potential source of infection.

The route of infection is discussed on p. 106, where it is pointed out that the respiratory and conjunctival routes may prove to be of practical importance.

Domestic animals and birds, and free-living animals, birds and invertebrates could, no doubt, play a part in the dissemination of infection within a herd, but as indicated on p. 117, they are more likely to be associated with infection with *S. typhimurium* and other serotypes than with *S. dublin*, and with infection in young calves than in older cattle. As before, the hazards of this form of spread must be kept in perspective against the major hazard presented by the bovine active carrier.

Persistence of infection in affected herds

This involves a number of factors, namely: the extent to which further cattle become infected; the persistence of infection within infected cattle and within any other host species that may become infected; the longevity of the organism after it is voided and its possible multiplication outside the animal body. Few data are available on the first point, but the fact that the clinical disease is traditionally endemic in certain areas suggests that when environmental conditions are suitable, and in the absence of control measures, infection may exist without limit in a given area or herd.

The persistence of the active carrier state in adult cattle was discussed on p. 107. For *S. dublin*, it may last for many years if not for life, but it is of shorter duration for *S. typhimurium*. The carrier state rarely persists in calves.

The persistence of *S. dublin* infection in farm rats was discussed on p. 116. Gibson (1958, 1961) suggested that the acquisition of *S. dublin* infection by rats might prolong the persistence of infection on a farm after the death or sale of infected cattle, but that this prolongation would probably be of limited duration. No doubt the same would apply to most cross-infections with this serotype. In contrast, one would expect *S. typhimurium* infection to persist almost indefinitely if it gained access to ducks.

Many authors have studied the longevity of salmonellae under various conditions, using cultures and naturally infected faeces, both from cattle and from other host species. As might be expected, their results show a wide variation according to the substrate, temperature, pH, bacterial flora and other attributes of the environment to which the salmonellae were exposed. Henning (1939) reported that *S. dublin* remained viable for at least 1069 days when infected bovine faeces were stored in the laboratory after they had been dried in the incubator for 48 h at 37 °C. Field (1948) took faeces from active carriers of *S. dublin* and found that the organism survived for 73–119 days on pasture and for 87 days in tap water. Gibson (1958, 1961) used similar material deposited on soil, on grass, on the surface of a stone wall and in jars of tap water and pond water, and recovered the organism for up to 150, 163, 307, 165 and

115 days, respectively. He suggested that *S. dublin* probably dies out within 6 months in faeces voided out of doors, but may remain viable for up to 10 months in faecal splashes on walls. However, calf faeces remained positive for a shorter time than those of adult cattle.

Other workers had reported a similar survival range for other serotypes. Lerche (1936, 1939) found that *S. typhimurium* survived in water for 15–20 days and for up to 12–16 months in earth. Josland (1951) also studied *S. typhimurium* and gave figures of 4–12 weeks for tap water, 12–24 weeks for pasture and 12–28 weeks for faeces. Watts & Wall (1952) found that this serotype survived for up to 119 days in water and for at least 200 days in soil. Felsenfeld & Young (1945) contaminated vegetables with cultures of various serotypes and reported survival periods of about 2–6 weeks at room temperature.

Similar observations have been offered during the period under review. Mair & Ross (1960) stated that in garden soil exposed to normal British weather, the survival time of *S. typhimurium* was probably not less than 280 days. Stewart (1962) made similar observations in Northern Ireland and found survival times of 110–160 days. Slavkov (1961) studied the longevity of *S. typhimurium* and 3 other serotypes in soil, both under laboratory and field conditions, and recorded survival for 120–150 days. Following an observation that bird faeces collected from live or dead vegetation yielded salmonellae more commonly than did samples collected from stones or soil, Steiniger (1961) investigated the survival of salmonellae in naturally infected bird faeces that were allowed to dry slowly on plant material before storage at room temperature. Some were still positive 28 months later. Van der Schaaf, van Zijl & Hagens (1962) infected feedingstuffs with salmonellae and found rather short survival times, of 2–28 days.

Under certain circumstances, salmonellae may not only survive after excretion but may also multiply. Von Garnier (1937) suggested that this occurred if wet feeding-stuffs containing sugar were contaminated with *S. typhimurium* in mouse faeces. Gauger & Greaves (1946) stated that at summer temperatures *S. typhimurium* would multiply in drinking water containing a little poultry mash, which they described as an excellent medium. Thompson (1953) showed that *S. paratyphi* B could multiply on damp flour sacks, and Gray *et al.* (1958) suggested that growth occurred in cattle food mixed with contaminated bone meal. Schaal (1961) demonstrated the growth of *S. dublin* in waste water at 18–20 °C, and Johne (1961) recorded the growth of both *S. dublin* and *S. typhimurium* in sterilized milk held at 9 °C.

It would, therefore, seem that the persistence of *S. dublin* infection on a given farm is chiefly governed by the extent to which the infection spreads among the herd and by the persistence of the active carrier state in individual animals. Cross-infection of other host species is probably less important than with *S. typhimurium* and other serotypes. The survival of the voided salmonellae is sufficiently long not to limit the spread of infection, either indoors or at pasture.

PUBLIC HEALTH CONSIDERATIONS

Because salmonellosis is one of the most common, widespread and important zoonoses of the temperate zones, the relationship between animal and human infection has become the subject of an extensive literature.

In Britain the chief public health significance of salmonella infection in cattle is that it provides an additional source of *S. typhimurium*, the serotype which is undoubtedly the chief cause of food poisoning in man. Cockburn (1962) reviewed the incidence of food poisoning in England and Wales for the 12 years of 1949–60 and reported that 44 % of the 69 869 known incidents were due to *S. typhimurium* and a further 16 % to other salmonellae. The figures for 1961 (Report, 1962) are similar; *S. typhimurium* was the presumed cause of 2503 (46 %) of the 5387 reported incidents of food poisoning, and for 64 % of the 3951 incidents of known causation. The figures for other salmonellae were 23 and 32 %, respectively. Twenty (91 %) of the 22 fatal cases of food poisoning were due to salmonellae, 7 of them to *S. typhimurium*.

S. dublin can also give rise to the food poisoning syndrome of acute vomiting and diarrhoea, and explosive outbreaks of this kind have been described by various workers including Conybeare & Thornton (1938), Tulloch (1939) and McCall (1953). It is, however, of minor importance compared with *S. typhimurium*. Apart from causing this kind of outbreak, *S. dublin* is sometimes responsible for meningitis and septicaemia in children (Smith, 1933) and occasionally for osteomyelitis (Miller, 1954) and localized abscess formations (Purnell, 1952; Davies, 1954). As previously mentioned, the isolates on which White (1930) based the original identification of *S. dublin* were from cases of this kind. However, even when these invasive infections are included, the incidence of *S. dublin* infection in man remains low. Thus in 1961 (Report, 1962) only 24 (0·7 %) of 3663 strains isolated from man in England and Wales were *S. dublin* whereas 2544 (69 %) were *S. typhimurium*.

Human infection with salmonellae can derive from cattle by contact, direct or indirect, with infected cattle on the farm, by contamination of the milk supply, or from contaminated or infected meat or offal. These will be discussed separately.

Human infection by farm contact

Gibson (1961) reported that investigation of 8 outbreaks of *S. typhimurium* infection in calves showed that in 3 outbreaks the infection had spread to the farm staff or their families. Dogs yielded the serotype on 2 of these premises and *S. dublin* on a third. Gibson suggested that the proportion of outbreaks that spread to man may be several times higher than is usually recognized, and that dogs may be of some importance in the transmission of infection from cattle to man, and especially to young children. Sirmon *et al.* (1962) described *S. typhimurium* and *S. enteritidis* infection that spread from carrier cows to affect calves and man, and Küng (1963) recorded the spread of *S. newport* infection from adult cattle to man. Five families were affected with 'long lasting intestinal grippe'. Angus & Barr (1963) reported an outbreak of *S. typhimurium* infection in a dairy herd. Samples taken from the farmer and his family showed 4 of them to be symptomless carriers. The reviewer suggests that many similar cases pass unrecognized, and that more attention should be paid to this method of entry of salmonella infection to the human population.

Milk-borne infection

As was discussed on p. 107, contamination of the milk supply can occur when a cow in the febrile stage of clinical salmonellosis excretes the organism in her milk. It seems that some active carriers may sometimes excrete infected milk but that this

is rare compared with the very real hazard of their infected faeces gaining access to the milk. There are many references to milk-borne outbreaks of human salmonellosis in Britain, and it is noteworthy that the first records of bovine salmonellosis in this country are to be found in the accounts of the outbreaks of human food poisoning to which they gave rise (Kerr & Hutchens, 1914; Savage, 1920).

Further outbreaks were reported during the period under review. Brief descriptions of the outbreaks occurring in England and Wales can be found in the annual reports of food poisoning published by the Public Health Laboratory Service. That for 1959 (Report, 1960b) gives details of 2 outbreaks of *S. typhimurium* infection. In one the source was not found. In the other the infection originated among calves and is believed to have been spread to the raw milk by the hands of the milker, who was also concerned with calf-rearing. At least 2 children who drank the milk had symptoms, and 1 adult was a symptomless excretor.

A more extensive outbreak was reported the following year (Report, 1961b) causing at least 60 cases of *S. typhimurium* infection among the customers and farm staff of a dairy selling unpasteurized milk. The organism was isolated from rectal swabs from 2 of the 36 cows in the herd and from a milk sample from one of them. The report for 1961 (Report, 1962) gives details of 3 outbreaks apart from those recorded by Parry (1962) and Knox *et al.* (1963) that are described below. One was due to *S. enteritidis* var. *jena*, which was isolated from the faeces of a sick cow on the farm supplying the milk. The other 2 outbreaks were due to *S. typhimurium*. Both were traced to infected cows. Parry (1962) reported an outbreak of *S. typhimurium* infection in Liverpool that was traced to unpasteurized tuberculin-tested milk from a local dairy farm. One of the cows of the milking herd had died from acute enteritis —presumably from the same cause, although no bacteriological examination had been made. Parry tabulated 14 milk-borne outbreaks of human salmonellosis recorded in Britain since 1951 and advocated that all milk should be heat-treated. Knox *et al.* (1963) reported an outbreak of *S. heidelberg* infection, also traced to unpasteurized tuberculin-tested milk. There were 56 human incidents. The infection was traced to a cow showing no clinical signs of the disease but excreting the organism in her milk. The veterinary aspects of this outbreak were recorded by Davies & Venn (1962) (see p. 108). Knox *et al.* (1963) gave a useful check list of 33 outbreaks of milk-borne human salmonellosis occurring in the United Kingdom between 1942 and 1961, and commented that whereas *S. dublin* was the commonest serotype in such outbreaks until 1950, it has since been replaced by *S. typhimurium* and that, more recently, other less common serotypes, such as *S. heidelberg*, have appeared. They too advocated the pasteurization of all milk.

The reviewer has seen no reference to a *S. dublin* outbreak in Britain during the period under review, but Schroeder & Dale (1960) reported one from Los Angeles, California, in which 47 human cases derived from unpasteurized milk. There was no history of clinical salmonellosis in the herd of origin but 3 of its 398 cows were found to be excreting the organism.

Taylor (1960) commented that although a number of outbreaks of human salmonellosis had been traced to infected milk in the past, milk and milk products were now rarely implicated in Britain because 95% of the milk was pasteurized, as was most cream and cheese. Outbreaks could still occur, however, if there were errors in

the pasteurization process. Moreover, the reports cited above show that unpasteurized T.T. milk, or milk taken raw by the farmer's family or staff, remains a potential source of infection.

Infection from slaughter animals

Infection can arise either from the slaughter of apparently healthy animals that are active or latent carriers of salmonellae, or from the slaughter of ailing animals, that is the so-called 'emergency slaughter' of the 'casualty animal'. Buxton (1957 *a*, *b*) described the casualty animal as a serious source of infection and stated that, with few exceptions, such cases should always be subjected to bacteriological examination. Certainly, if undetected, they may not only cause infection in persons handling or consuming the meat and offal or feedingstuffs prepared from them, but may also give rise to gross contamination of other carcasses and products. Because of this, Galbraith *et al.* (1961) advocated the prohibition of the slaughter of sick animals in abattoirs handling meat for human consumption. Clinically normal active and latent carriers constitute a more insidious but more serious hazard. The routine bacteriological examination of large numbers of clinically normal slaughter animals is usually impractical, and control measures must therefore be based on improved abattoir hygiene (Buxton, 1957 *a*, *b*). Norval (1961) discussed the details of the necessary hygienic precautions.

On p. 102 the continued incidence of salmonella infection in slaughter cattle has already been indicated, but mention should be made here of the high incidence found in apparently healthy adult slaughter cattle in Costa Rica by Arroyo & Bolaños (1960) who isolated salmonellae of 8 serotypes from the organs of 26 (13 %) of 195 animals, and of the serious problem of *S. typhimurium* infection in New Zealand investigated by Nottingham & Urselmann (1961) following reports such as that by Kallings, Laurell & Zetterberg (1959) that New Zealand veal was causing human infection in countries to which it was exported. Other references of interest include those of Ekstam (1961) who studied cross infection between calves and man in abattoirs in Sweden, and of Sandbu (1960) who reported that 17 (74 %) of 23 calves that had survived an outbreak of *S. typhimurium* infection yielded the organism from their viscera although the faeces were negative. The surfaces of the musculature of 5 were also positive.

Reference has already been made to the important paper of Anderson *et al.* (1961), who investigated a series of outbreaks of human food poisoning in south-east England due to *S. typhimurium* phage type 20 *a*. They obtained evidence strongly suggesting calf meat as the source of the infection in at least three-fifths of the incidents and found that up to 13 % of apparently normal calves in the abattoirs concerned were excreting *S. typhimurium* of the same phage-type. There were strong indications of a build-up of infection between the farm and the slaughter point. The authors emphasized the value of phage-typing in an investigation of this kind, both in linking up scattered human cases which would not otherwise have been recognized as being epidemiologically related, and also in providing the clue as to the likely source of infection.

Harvey & Phillips (1961) isolated salmonellae of 21 serotypes from 111 (40·5 %) of 274 swabs from the gullies of abattoirs in which pigs and sheep were killed as well

as cattle. They found that the phage-types of *S. typhimurium* isolated from the abattoirs not infrequently corresponded in timing with the strains isolated from human infections, and they concluded that some of the less dramatic, but widespread, outbreaks of human salmonellosis might originate from butchers' meat. In a subsequent investigation Harvey, Price, Bate & Allen (1963) reported a correlation between the isolation of *S. typhimurium* phage-type 12 from scattered cases of human food poisoning in south Wales and from the drains of 2 large abattoirs. The organism was also isolated from the floor drains of 15 (28 %) of 54 butchers' shops and bakehouses. Again, the phage-typing technique was invaluable in associating the scattered incidents and indicating the source of infection.

In contrast to these conclusions, Jones, Bennett & Ellis (1961) stated that the serotypes isolated from 200 sewer swabs from the Coventry public abattoir were in the main dissimilar to those causing overt infections in the human population of the city during the same 12-month period. This, however, may have been because whereas Harvey and his co-workers compared the occurrence only of those strains known to affect both animals and man, Jones *et al.* compared all the isolates from the 2 sources, a method that would allow the related incidents to be overshadowed by human cases originating from other sources. Galbraith (1961) and Galbraith *et al.* (1961) described investigations into the epidemiology of *S. saint-paul* and *S. heidelberg* infection in man. Both investigations disclosed a complex epidemiological chain involving calves and pigs and probably originating from imported feedingstuffs.

It is evident that recent epidemiological studies have provided valuable additional evidence of the relationship between the salmonella infections present in slaughter cattle and other slaughter animals and in the human population. The phage-typing technique has been especially valuable in this respect.

The resistance of salmonellae to antibiotics

In view of the importance of salmonella infection in man it is disquieting to note that several workers have reported a significant increase in the proportion of salmonellae that exhibit resistance to tetracyclines and to chloramphenicol. Thus Ramsey & Edwards (1961) reported that whereas only 5 % of 100 strains of *S. typhimurium* isolated from man in 1956–57 were resistant to tetracyclines, 14 % of 158 strains isolated in 1959–60 were resistant. Manten, Kampelmacher & Guinée (1961) reported a slight decrease between 1958 and 1960, but a significant increase between then and 1961 (Manten, Kampelmacher & Guinée, 1963) when 6 (1 %) of 511 strains of *S. dublin* and 33 (18 %) of 183 strains of *S. typhimurium* isolated from cattle were resistant to tetracyclines. McWhorter, Murrell & Edwards (1963) also found that *S. typhimurium* showed a higher incidence of resistance to chlortetracycline than did other serotypes. Of 48 strains from cattle, 45 (94 %) were resistant. They also stated that the incidence of resistance shown by strains isolated from man had increased from 14 % in 1959/60 to 38 % in 1962. Strains from fowls did not show this increase.

Tetracycline preparations have been used in the U.S.A. to prolong the shelf life of poultry carcasses and it has been suggested that in hot climates they should also be used for beef (Ginsberg, Hill & Grieve, 1957; Ginsberg, Reid, Grieve & Ogonowski, 1958). However, Hobbs *et al.* (1960) reported that when poultry carcasses that had

been treated by immersion in slush ice containing chlortetracycline were later held at 22 °C, a strain of *S. typhimurium* resistant to this antibiotic was able to outgrow the spoilage organisms. The presence of such resistant strains would therefore be of considerable public health significance in any countries that adopted a process of this kind. The treatment of human salmonellosis is not dependent upon the tetracyclines and chloramphenicol. Even so, it would seem wise to keep this subject under review by means of periodic surveys, as suggested by Ramsay & Edwards (1961).

<div align="center">LABORATORY DIAGNOSIS</div>

This falls under 2 headings, firstly the confirmation of suspected clinical salmonellosis and its differentiation from such conditions as coccidiosis and bracken poisoning (Field, 1959) and, secondly, the identification of the carrier animal.

Confirmation of clinical disease

In the live animal, confirmation is normally sought by cultural examination of the faeces. Field (1959) pointed out that in adult cattle faecal culture may give negative results in the early febrile stage of the disease, before the onset of dysentery, and Gibson (1961) stated that because calves tend to excrete the organism intermittently it may be necessary to sample them several times, or to examine the batch rather than the individual, in order to establish a diagnosis.

Many workers have introduced new or modified media for the enrichment, isolation and presumptive diagnosis of salmonellae, and the choice between these, and their method of use, remains very much a matter of personal experience and preference. Thus the selection of media in North America tends to differ from that used on the mainland of Europe and also from that used in Britain, where the combination of a selenite broth and desoxycholate citrate agar (DCA) recommended by Smith (1952) and by Field (1959) is still widely used.

A discussion of the various new cultural methods that have been advocated during the period under review is outside the scope of this paper, but it is of interest to note that Harvey & Phillips (1961) and Harvey & Price (1962) stated that for certain types of material enrichment is best achieved by the use of selenite broth incubated at 43 °C instead of at the conventional 37 °C, and that multiple subculture of the broth aids the separation of the various serotypes that may be present in a single sample. Dixon (1961) also advocated incubation at 43 °C as an aid to rapid diagnosis. Köser & von Sprockhoff (1960) found that the isolation of *S. dublin* and *S. typhimurium* was delayed or prevented when therapeutic doses of antibiotics had been given.

During recent years much attention has been given to the technique of staining micro-organisms using their specific antibodies 'labelled' with a fluorescent dye such as fluorescein isocyanate. Where applicable, this technique provides a specific diagnosis well before the results of cultural examinations are available. Thomason, Cherry & Edwards (1959) applied the method to the identification of salmonellae in faecal smears, but reported that cross-reactions with other organisms were a serious problem. Gizatullin, Akhmerov, Busygin & Barskov (1961) and Iganat'eva (1961) claimed success for the identification of *S. cholerae-suis* by this method and, as mentioned on p. 108, Arkhangel'skii & Kartashova (1962) stated that the method was satisfactory for demonstrating salmonellae in artificially contaminated milk

when 100000 or more organisms per ml were added. Cultural methods were superior for smaller numbers. It seems that the technique warrants further study as a potential diagnostic tool, both for the rapid diagnosis of clinical disease and also for the screening of large numbers of specimens in abattoirs where conventional cultural methods might be impractical. Serological examination can be used retrospectively to establish a diagnosis and may be useful in outbreaks in calves if excretion of the organism is intermittent or scanty (Gibson, 1961).

Diagnosis of the carrier state

The reports of Galbraith (1961), Harvey & Phillips (1961) and Harvey *et al.* (1963) illustrate the value of the sewer-swab technique (Moore, 1948) for investigating the salmonella status of a large number of animals, such as cattle passing through a city abattoir. The technique obviously effects a great saving in time and media compared with the sampling of individual animals or carcasses. Indeed, Harvey & Phillips (1961) found it more effective than the examination of meat specimens.

Some workers, including Lovell (1953), have advocated that the individual members of a suspect herd should be examined serologically, and that faecal culture should be reserved for those with high blood agglutinin levels. Geurden *et al.* (1960) suggested that the bacteriological identification of carrier animals was hampered by the widespread use of antibiotics and coccidiostats and advocated that more sensitive serological tests should be developed. The reviewer, however, suggests the opposite approach, as did Field (1959) and Stellmacher (1963*b*), by which the whole herd is examined culturally using rectal swabs. Serology is then normally reserved for those animals yielding positive swabs, although a more general serological examination is useful if facilities on the farm and in the laboratory allow this. Field (1959) pointed out that isolation of salmonellae at a single test is not sufficient to classify a cow as an active carrier because many cows kept in close contact with an active carrier or with a clinical case will show transient excretion of the organism. He advocated that 3 tests should be made at intervals of 7–14 days and that only those animals giving 3 successive positive results should be classified as active carriers.

It is of interest to note that Arkhangel'skii and Kartashova (1962) described a milk ring test using a *S. enteritidis* antigen stained with haematoxylin. Compared with the collection and examination of blood samples, a method of this kind would effect a considerable saving in time and materials. Another promising development is one in which particles of polysterene latex or of bentonite are sensitized with bacterial extracts to give either a monovalent or polyvalent somatic antigen (Magwood & Annau, 1961; Diena, Wallace & Greenberg, 1963). The possible application of fluorescent microscopy in abattoir control work has already been referred to above.

TREATMENT

Treatment of adult cattle

Field (1959) pointed out that treatment of adult cattle is of little value if the recovered animals remain carriers and a source of infection to others. He commented, moreover, that the reports then available provided no conclusive evidence that salmonellosis in adult cattle could be successfully treated by therapeutic means. Little has been reported since then to modify this assessment with regard to *S. dublin*

infection, and the reviewer suggests that clinically affected animals should be slaughtered—preferably at a rendering plant—as soon as the diagnosis is confirmed. It is true that Amor & Hopkins (1962) reported that treatment with a mixture of furazolidone and nitrofurazone (Bifuran; Smith, Kline & French Ltd.) seemed extremely effective, but as pointed out by Field (1959) it is impossible to assess the effect of treatment on such small numbers.

This radical approach may be less necessary when infection is with *S. typhimurium* or other serotypes, as the carrier state produced by these is apparently of shorter duration (Report, 1959). Küng (1963) treated *S. newport* infection in cattle with intravenous chloramphenicol (3000–7000 mg) and claimed good results, both with clinical cases and with carriers. Haxby (1961) advocated a combination of intramuscular and oral neomycin for the treatment of *S. typhimurium* infection. He gave 3 g intramuscularly and 2 g twice daily by mouth. This was repeated for 4 days Riddell, Norval & Anderson (1959) reported an outbreak in which 67 of 400 cows were. excreting *S. typhimurium* and in which the faeces became negative for salmonellae after treatment with a nitrofuran. However, one cannot judge whether this was due to treatment or to spontaneous remission as no untreated controls were kept. Rude (1963) reported that clinical disease due to *S. typhimurium* was generally refractory to treatment, both in calves and in adult cattle.

In passing, it is of interest to note that Williamson (1961) claimed good results for an organic arsenical given in the drinking water for the treatment of *S. typhimurium* infection in sheep, and that Janssen & van Lipzig (1961) considered furazolidone effective for the treatment of *S. typhimurium* infection in 18-month-old foals.

Treatment of calves

As was stated on p. 107 calves that recover from clinical salmonellosis rarely remain excretors of the organism. The objections raised in the previous subsection to the attempted treatment of adult cattle do not, therefore, apply to calves.

The references prior to 1960 were summarized by Gibson (1961), who emphasized the need for nursing measures in addition to specific therapy. Hartigan (1960) also advocated 'adequate supportive therapy' in addition to antibiotics and sulphonamides. Haxby (1961) used a combination of intravenous, intramuscular and oral neomycin to treat calves with *S. dublin* infection and reported that 12 of 13 were cured within 48 h of developing per-acute symptoms. He gave 2 g intravenously on the first day, followed by 1 g intramuscularly once daily for the next 3 days. One gramme was given orally twice daily for the same 4-day period. Wood, Raju & Williams (1962) infected calves experimentally with *S. typhimurium* and treated them with furaltadone at the daily rate of 6 mg/lb body-weight. The non-medicated calves died within 5 days whereas the treated ones showed only transient diarrhoea.

The observations noted on pp. 123–4 on the development of resistance to certain antibiotics suggest that sensitivity tests should be carried out during the early stages of an outbreak in order to ascertain whether the strain in question is sensitive to the drug chosen for treatment.

PREVENTION AND CONTROL

The chief practical interest in preventive measures is in respect of the disease in young calves, and especially in calves gathered from various premises for large-scale intensive rearing, as these are the age groups and circumstances in which the disease is most likely to cause heavy losses and to become a recurrent problem. This is in marked contrast to the sporadic nature usually shown by the disease among older cattle. However, many of the following remarks will be equally applicable to various age groups and systems.

The preventive measures available can be divided arbitrarily into short- and long-term measures.

Short-term measures

These comprise the avoidance of introduction of infection; avoidance of the transfer of infection from one batch to another; avoidance of intercurrent disease or of errors or methods of management that would favour the development of clinical salmonellosis, and the use of preventive medication and of vaccines.

On pp. 110–117 the introduction of infection is discussed, and it follows from the discussion there that control measures should include careful selection of purchases, the isolation of newly purchased animals and also of all young calves—the latter being the animals most susceptible to infection—the control of rats and mice, and the screening of calf houses against these and against wild birds. Contaminated feeding-stuffs do not seem to constitute a major hazard and are more likely to produce subclinical rather than clinical infection. The risk is greatly reduced if they are used in pelleted form (Report, 1961).

The avoidance of cross-infection calls for adequate routines of cleaning and disinfection and a sound lay-out for the whole enterprise. The latter was described by Manton (1963). An all-in-all-out system, as practised by the broiler poultry industry, has much to commend it.

Gibson (1961) mentioned the difficulty of buying-in large numbers of calves free of disease and suggested that, as a working principle, they should be obtained by as direct a route as possible, avoiding those that had been exposed in several markets or collecting centres. Manton (1963) described how calves entering a vaccine manufacturer's premises were examined culturally for salmonellae and were retained in individual pens until they were found to be free from infection. This method was very successful. In the reviewer's experience, furazolidone and tetracyclines are commonly used prophylactically in young calves, although the practice has received little mention in the literature. Tetracyclines appear to be preferable for this purpose because of their less specific mode of action. Moreover, Blaxland (1960) has reported that in poultry the prophylactic use of furazolidone can give rise to resistant strains that may subsequently cause losses.

In the past, a number of workers including Gribanov (1937), Pellissier, Trinquier & Troquereau (1948) and Henning (1953b, 1954) have advocated vaccination for the prevention of salmonellosis in calves. Further reports have been published during the period under review. Bandaranayake & Thambiaiyah (1961) reported that 3 outbreaks of *S. dublin* infection in Ceylon were quickly controlled by an oil adjuvant

autogenous vaccine. As a first step all animals under 6 months of age were vaccinated. Later all newborn calves were vaccinated during the 1st week of life. Malyavin & Gol'denman (1961) investigated the transfer of agglutinins from vaccinated cows, via the colostrum, to their calves, but Stellmacher (1963b) stated that maternal vaccination was not an effective way of protecting young calves against *S. dublin*. Hartigan (1960) reported that a commercial polyvalent vaccine had proved ineffective in Ireland, and a report from Bechuanaland (Report, 1960) stated that although extensive use was made of vaccine it was inadequate in the face of the poor sanitary conditions and husbandry system found there. Boccadoro & Veneroni (1962) studied experimental infection with *S. dublin* in rabbits, and advocated the widespread use of oral vaccines. Pritulin (1961) followed his previous study of the respiratory route of infection (Pritulin, 1959) by an investigation of the use of this route for immunization, using an aerosol preparation and face masks. He claimed that this was more effective than either subcutaneous or oral administration.

Long-term measures

In poultry it has been found that certain breeds or strains are more resistant than others to salmonella infection (Smith, 1956; Hutt & Crawford, 1960). The demonstration and exploitation of similar genetic differences in cattle would provide one long-term method of combating salmonella infection, but as yet this has not been explored.

A second long-term approach depends upon the identification and removal of active carriers from the adult herd, since, as has been shown in the preceding pages, such carriers constitute the chief source of *S. dublin* infection in calves and older cattle and are probably one of the more important sources of *S. typhimurium* infection. This approach was advocated by Proescholdt (1931) and Manninger (1939). One method of exercising this form of control is to make clinical salmonellosis of adult cattle a notifiable disease. Bacteriological confirmation of the diagnosis is then followed by examination of the rest of the herd for active carriers, and the slaughter of animals whose faeces are consistently positive for salmonellae. Measures of this kind were in force in Germany in the 1940's (Anon. 1943) and were advocated by Beijers (1948) and by Clarenburg & Vink (1949), who described the application of a similar system in one province of Holland. Roemmele & Westphal (1956) gave details of this method of control, and suggested that at least 3 months were required to detect all the carriers in a herd. Müller (1957) attributed the decreased incidence of *S. dublin* infection in calves in Denmark to the detection and slaughter of carrier cows. Field (1959) also described this approach and stated that the flesh of slaughtered carriers could be used for human consumption provided certain precautions were taken at the time of slaughter. Harms (1959) suggested that regular faecal examinations should be made in tuberculin-tested herds.

Similar reports and recommendations have been published during the period under review. Schroeder & Dale (1960) described how active carriers of *S. dublin* were removed from a dairy herd. Stellmacher (1963b) advocated repeated examination with rectal swabs as the method of choice for eradicating *S. dublin* from a herd, and Schaal (1963) stated that the culling of active carriers, together with hygienic and managemental measures, was effective in controlling *S. typhimurium* infection in a herd. Dijkstra (1961) described the eradication of calfhood infection with *S. dublin*

from 36 of 37 infected herds following the removal of carrier cows and more strict isolation of the calves. Preston (1963) suggested that the possibility of an eradication programme deserved consideration in Britain.

It is seen that the individual owner can do much to minimize his losses from salmonellosis. Attention to management and avoidance of travel-weary market calves are important. Current reports on the efficiency of vaccines are equivocal, but the production of an efficient vaccine would undoubtedly be of great value to the industry. There is little doubt, however, that eradication should be the aim when *S. dublin* infection occurs in a self-contained herd. The same approach could be followed by groups of farmers wishing to establish a co-operative calf-rearing enterprise. Similar action against adult carriers of *S. typhimurium* would reduce what is probably the chief source of infection with this serotype in calves as well as reducing one of the sources contributing to infection in man.

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