by chymosin during cheese production and leads to the micelles sticking together to form a coagulum (see Section 9.6).

The balance of the protein in milk is made up of the whey proteins. These mainly comprise the compact globular proteins β-lactoglobulin and α-lactalbumin but also a number of blood-derived proteins such as serum albumin and immunoglobulins. The latter are present at higher levels in colostrum where they presumably confer some resistance to infection in the newborn calf.

5.2.2 Microflora of Raw Milk

Its high water activity, moderate pH (6.4–6.6) and ample supply of nutrients make milk an excellent medium for microbial growth. This demands high standards of hygiene in its production and processing; a fact recognized in most countries where milk was the first food to be the focus of modern food hygiene legislation.

Milk does possess a number of antimicrobial features (discussed in Section 3.2.4), present either to protect the udder from infection or to protect the newborn calf. Generally these are present at too low a concentration in cow's milk to have a very marked effect on its keeping quality or safety. In some cases the antimicrobial activity is antagonized by other milk constituents such as the effect of citrate and bicarbonate on lactoferrin activity. Stimulation of lactoperoxidase activity through the addition of exogenous hydrogen peroxide has been investigated as a means of preserving raw milk in developing countries where ambient temperatures are high and refrigeration is not often available. In one trial in Africa, use of this technique increased the proportion of samples passing the 10 minute resazurin quality test from 26% to 88%.

Three sources contribute to the micro-organisms found in milk: the udder interior, the teat exterior and its immediate surroundings, and the milking and milk-handling equipment.

Bacteria that get on to the outside of the teat may be able to invade the opening and thence the *udder interior*. Aseptically taken milk from a healthy cow normally contains low numbers of organisms, typically fewer than 10^2–10^3 cfu ml^{-1}, and milk drawn from some quarters may be sterile. The organisms most commonly isolated are micrococci, streptococci and the diptheroid *Corynebacterium bovis*. Counts are frequently higher though due to mastitis, an inflammatory disease of the mammary tissue, which is a major cause of economic loss in the dairy industry. In England and Wales, where it has been estimated to cost the industry around £90 million annually, about 1–2% of cows have a clinical infection at any one time. In the early acute stage of illness the bacterial count in mastitic milk can exceed 10^8 cfu ml^{-1} and macroscopic changes are often visible in the milk. Mastitis is also diagnosed by the presence of high numbers of polymorphonuclear leukocytes which can rise to levels of 10^7 ml^{-1} in infected milk.

In addition to acute mastitis, a substantial proportion of the national dairy herd is subclinically infected. In these cases there may be no obvious signs of infection yet the causative organism can be present in the milk at about 10^5 cfu ml^{-1} and will contribute to an increase in the overall count of bulked milk.

Many organisms can cause mastitis, the most important being *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, *Pseudomonas aeruginosa* and *Corynebacterium pyogenes*. Several of these are potential human pathogens and a number of other human pathogens such as *Salmonella*, *Listeria monocytogenes*, *Mycobacterium bovis* and *Mycobacterium tuberculosis* are also occasionally reported.

Infected cows are treated by injection of antibiotics into the udder. Milk from these cows must be withheld from sale for several days following treatment because antibiotic residues can cause problems in sensitive consumers and inhibit starter culture activity in fermented milks. Attempts to control mastitis by good milking hygiene, use of a disinfectant teat dip after milking and an antibiotic infusion at the end of lactation have helped to reduce streptococcal and staphylococcal infections but have had little success in preventing *E. coli* mastitis.

The *udder exterior and its immediate environment* can be contaminated with organisms from the cow's general environment. This is less of a problem in summer months when cows are allowed to graze in open pasture and is worst when they are housed indoors and under wet conditions. Heavily contaminated teats have been reported to contribute up to 10^5 cfu ml^{-1} in the milk. Contamination from bedding and manure can be a source of human pathogens such as *E. coli*, *Campylobacter*, and *Salmonella* and *Bacillus* species may be introduced from soil. Clostridia such as *C. butyricum* and *C. tyrobutyricum* can get into milk from silage fed to cows and their growth can cause the problem known as late blowing in some cheeses.

A number of measures can be taken to minimize milk contamination from the udder exterior and considerable advice on this topic is available to dairy farmers. Some of the recommendations made by the Milk Marketing Board, formerly the principal purchaser of milk in England and Wales, included:

(1) providing enough clean bedding and replacing it as necessary;
(2) removing slurry (faeces and urine) from concrete areas at least twice daily;
(3) preventing muddy areas wherever possible;
(4) shaving udders and trimming tails;

(5) washing teats with warm water containing disinfectant and drying individually with paper towels;
(6) keeping the milking parlour floor clean during milking;
(7) thoroughly cleaning teat cups if they fall off during milking and discarding foremilk.

Although such procedures certainly improve the microbiological quality of milk, economic constraints such as increasing size of individual dairy herds and decreased manning levels in milking parlours encouraged their neglect. The introduction of total bacterial count as a basis for payment in 1982 provided an incentive for their more zealous application and led to a marked decline in bacterial count of milk (see below).

Milk-handling equipment such as teat cups, pipework, milk holders and storage tanks, is the principal source of the micro-organisms found in raw milk. As the overall quality of the milk decreases so the proportion of the microflora derived from this source increases. Milk is a nutritious medium and, if equipment is poorly cleaned, milk residues on surfaces that are frequently left wet will act as a focus for microbial growth which can contaminate subsequent batches of milk. Occasional neglect of cleaning and sanitizing procedures is usually less serious since, although it may contribute large numbers of micro-organisms to the product, these tend to be fast growing bacteria that are heat sensitive and will be killed by pasteurization. They are also sensitive to sanitizing practices used and will be eliminated once effective cleaning is resumed. If cleaning is persistently neglected though, the hydrophobic, mineral-rich deposit known as milkstone can build up on surfaces, particularly heated ones. This will protect organisms from sanitizers and allow slower growing organisms to develop such as micrococci and enterococci. Many of these are thermoduric and may not be removed by pasteurization.

To encourage farmers to apply the available advice on animal husbandry practices, milking procedures, types and design of equipment and cleaning schedules which contribute to good bacteriological quality milk, the Milk Marketing Board (MMB) in England and Wales introduced in 1982 a system of paying farmers based on the total bacterial count (TBC) of their milk. Similar schemes have been introduced in a number of countries but details of the MMB's scheme are presented as Table 5.3. For four months prior to introduction of the scheme, farmers were notified of the TBC count of their milk and in anticipation of its start a dramatic fall in the count was noted (Figure 5.2). Now more than 76% of the milk produced in England and Wales falls into Band A with a mean count of 1.7×10^3 cfu ml^{-1}. The Milk Marketing Board no longer exists as the monopoly purchaser of farm milk in England and Wales, but the bodies that replaced it recognized the value of a payment scheme which includes microbiological quality and have retained similar systems.

Table 5.3 *Milk Marketing Board (England and Wales) total bacterial count payment scheme*

Grade	*Count (cfu ml^{-1})*	*Price adjustment (pence l^{-1})*
A	$<2\times10^4$	+0.23
B	$>2\times10^4$ but $<10^5$	0
C_1	$>10^5$ but no price deduction in previous 6 months	−1.5
C_2	$>10^5$ and Grade C_1 produced in previous 6 months	−6.0
C_3	$>10^5$ and Grade C_2 or C_3 deduction has been applied	−10.0

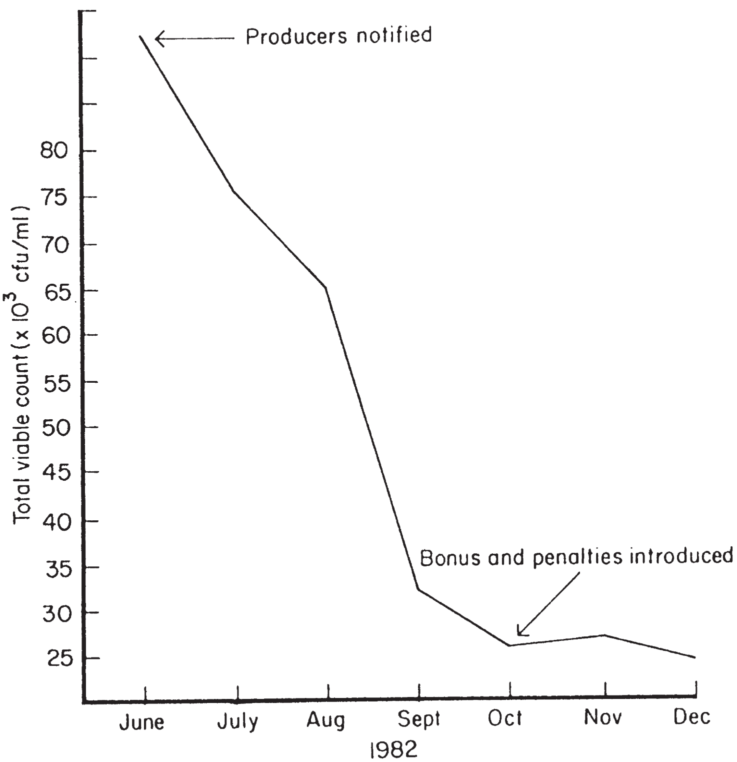

Figure 5.2 *Raw milk counts and the bonus payments scheme. Reproduced from 'Micro-organisms in Agriculture', SAB Symposium Series No. 15*

In most developed countries milk is chilled almost immediately after it issues from the cow and is held at a low temperature thereafter. It is stored in refrigerated holding tanks before being transported by a refrigerated or insulated lorry to the dairy where it is kept in chill storage tanks until use. Throughout this time, its temperature remains below

7 °C and the only organisms capable of growing will be psychrotrophs. There are many psychrotrophic species, but those most commonly found in raw milk include Gram-negative rods of the genera *Pseudomonas, Acinetobacter, Alcaligenes, Flavobacterium*, psychrotrophic coliforms, predominantly *Aerobacter* spp., and Gram-positive *Bacillus* spp.

One consequence of the current extensive use of refrigeration and the change to a microflora dominated by psychrotrophs is that traditional tests for the microbiological quality of milk based on the reduction of a redox dye such as methylene blue or resazurin have become obsolete. Psychrotrophs tend to reduce these dyes poorly and the tests are not very sensitive to low numbers of bacteria.

5.2.3 Heat Treatment of Milk

Proposals for the heat treatment of milk were made as early as 1824, forty years before Pasteur's work on the thermal destruction of microorganisms in wine and beer. When milk pasteurization was introduced by the dairy industry around 1890, it was as much to retard souring as to prevent the spread of disease. This had become an important commercial requirement since large quantities of milk were now being transported by rail into the large cities rather than being produced locally in cramped and insanitary cowhouses.

Milk has long been recognized as an agent in the spread of human disease and within a few years it was appreciated that pasteurization was also providing protection against milk-borne disease. Nowadays it is safety rather than spoilage considerations which determine the minimum legal requirements for pasteurization.

Originally the main health concerns associated with milk were tuberculosis caused by *Mycobacterium bovis* and *Mycobacterium tuberculosis* (see Section 7.10) and brucellosis caused by *Brucella* spp. (see Section 7.3). In some parts of the world milk is still a significant source of these infections but in the UK and some other countries they have now been effectively eliminated from the national dairy herd by a programme of regular testing and culling of infected animals. Such programmes must be constantly maintained to be effective and there have been occasional problems. Initiatives such as the culling of badgers, thought to be a reservoir of *M. bovis*, have been the subject of some controversy and in 2002 there was an outbreak of brucellosis in a dairy herd in Cornwall, although this was the first recorded in England for ten years. Enteric pathogens such as *Salmonella* and *Campylobacter* are still however prevalent in raw milk and pasteurization remains the most effective measure for their control.

The four types of heat treatment applied to milk are described in Table 5.4. Specification of pasteurization temperatures to the first decimal