Fate of Nonpathogenic and Enteropathogenic \textit{Escherichia coli} During the Manufacture of Colby-like Cheese

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ABSTRACT

Pasteurized whole milk was artificially contaminated with 100 to 1000 \textit{Escherichia coli}/ml and was used to manufacture Colby-like cheese. Some cheeses were made so their composition differed from that of normal Colby cheese. Cheeses were cut in half and stored at 3°C and 10°C. \textit{E. coli} was enumerated by surface-plating of samples on Trypticase Soy Agar (TSA) with an overlay of Violet Red Bile Agar (VRB). \textit{E. coli} increased by 100 to 1000-fold, depending on the strain, to about 1 x 10^4/g of curd, in most instances, by the end of the cook (3.5-3.9 h). After this point numbers of \textit{E. coli} in cheeses generally decreased over a period of weeks. One strain of enteropathogenic \textit{E. coli} (EEC) could not be detected after 4 weeks, and another (in all but one instance) after 6 weeks. However, EEC in one lot of cheese persisted at numbers in excess of 1 x 10^5/g after 12 weeks of refrigerated storage. EEC survived at low levels (<350/g) for many weeks in one instance. Cheeses of poor quality (high moisture and pH) were made to assess the effects of improper manufacture on survival of \textit{E. coli}. In these cheeses, \textit{E. coli} eventually reached numbers in excess of 1 x 10^8/g and persisted for many weeks at high numbers. Survival of \textit{E. coli} in Colby-like cheese appeared to be influenced by pH, salt and temperature; pH seemed to have the greatest effect on survival of the bacterium.

Enteropathogenic \textit{Escherichia coli} (EEC) can be defined as any strain of \textit{E. coli} that has the potential to cause diarrheal disease. These strains may be toxigenic, invasive, or possess unknown pathogenic mechanisms. Enterotoxigenic \textit{E. coli} elaborate one or more enterotoxin and may produce a chlorea-like syndrome, traveler’s diarrhea or infantile diarrhea. Enteroinvasive \textit{E. coli} cause a shigella-like diarrhea after an invasive infection of the colon. Documented outbreaks of EEC diarrheal illness around the world have been associated with such foods as meat and meat products, fish, poultry, milk and dairy products, vegetables, baked products, rice formulations, coffee substitutes and water (U.3). Presence of EEC in cheese has become a matter of concern since 1971 when at least 387 persons in 107 separate episodes were stricken with gastroenteritis after ingestion of EEC-contaminated Camembert cheese imported from France (U.2). Counts on cheese samples manufactured on the production days in question revealed the presence of 10^5 to 10^7 enteroinvasive \textit{E. coli} 0124/g of cheese (U.2). Since this outbreak occurred, several studies have been done to characterize survival of EEC in fermented skimmilk (U.4), Camembert cheese (U.6,15) and brick cheese (U.7).

Glatz and Brudvig (U.8) recently tested commercial cheese samples and found \textit{E. coli} in 41% of intermediate-moisture cheeses. The high incidence of \textit{E. coli} in these cheeses suggests that they could be a vehicle for transmission of EEC. Thus it was thought that Colby cheese, an intermediate-moisture cheese, might be a potential vehicle for transmission of EEC. Colby cheese has a shorter shelf life than does Cheddar cheese because Colby cheese generally has a higher moisture content, higher pH, and lower salt concentration. These properties may also permit greater survival in the cheese of any EEC present in the milk supply used for cheesemaking. Hence the study to be reported in this paper was undertaken to determine growth and survival of nonpathogenic and enteropathogenic strains of \textit{E. coli} in Colby-like cheeses under different conditions of manufacture and storage. Many of the cheeses were made so their composition was abnormal, which might favor survival and even growth of \textit{E. coli}.

MATERIALS AND METHODS

Cultures

Cultures of enteropathogenic \textit{E. coli} used included two enterotoxigenic strains, B4 and H10407, and two enteroinvasive strains, 4608 and 0128B12 (NY), obtained from the Food and Drug Administration. Nonpathogenic \textit{E. coli} strain K12 was obtained from the Department of Bacteriology at the University of Wisconsin. Cultures of \textit{E. coli} were grown in Trypticase Soy Broth (Difco) at 37°C for 24 h before they were used as inoculum. Enough of the broth culture was added to coliform-free pasteurized milk to provide between 100 and 1000 \textit{E. coli}/ml. The starter culture was \textit{Streptococcus lactis} C-6 obtained from the Marshall Division of Miles Laboratories in Madison, Wisconsin.
This culture was incubated at 21°C in autoclaved (121°C, 15 min) skim milk for 20-22 h before cheesemaking; the skim milk was coagulated under these conditions. A 2.0% inoculum of starter culture was used.

**Manufacture of cheese**

Cheese was made from 5 L of pasteurized coliform-free milk in sanitized stainless steel pans. Temperature was adjusted in a water bath (Thelco Model 84, Precision Scientific Co.). Milk was heated to 32.8°C. One hundred ml of starter culture were added as was 5 ml of the appropriate dilution of *E. coli* in an aqueous solution of 2% sodium citrate. Mixing was accomplished with a stirrer (T-Line, Model #105, Talboys Instrument Corp.) attached to a variable autotransformer (type 2PF1010 or type 3PN1010, Staco Inc.). One hour after addition of the starter culture, 1 ml of rennet, either Maryzyn II or Maryzyn Single Strength Microbiological Rennet (Marshall Division-Miles Laboratory Inc.), was added. Clotted milk was cut 45 min later, and this was followed by gradual heating to 38.9 to 40.0°C over 30 min. The temperature was maintained at 38.9 to 40.0°C for 1 h, after which whey was drained to just above the curds in the pans (cheese made with nonpathogenic *E. coli K-12* was cooked for 1 h and 15 min). Cold coliform-free tap water was added until the temperature of the curd plus liquid reached 32.8°C (33.3°C when cheese was made with *E. coli K-12*). The curd was agitated for 28-53 min to remove lactose and hence to insure a curd of high pH. Curds, which were maintained at 38.9°C for 1 h, after which whey was drained to just above the curds in the pans (cheese made with nonpathogenic *E. coli K-12* was cooked for 1 h and 15 min). Cold coliform-free tap water was added until the temperature of the curd plus liquid reached 32.8°C (33.3°C when cheese was made with *E. coli K-12*). The curd was agitated for 11-20 min (Table 1) after addition of water and then the liquid was removed. (During manufacture of poor quality cheeses curd was agitated 28-53 min to remove lactose and hence to insure a curd of high pH.) Curds, which were maintained at 32.8°C (33.3°C for cheese made with *E. coli K-12*) were stirred with sanitized instruments until the pH of the curd reached about 5.6 (in some experiments this pH value varied, see Table 1). After this, cheese was salted three times. Approximately equal amounts of salt were added each time. During 5-min intervals between salting, curd was stirred thoroughly. A total of 12 g of salt was added. Salted curds were then hooped and pressed. The following day cheese was cut in half and one portion was stored at 3±1°C and the other at 10±1°C (Ambi-Hi-Lo Chamber, Lab-Line Instruments Inc. or Incubator Model #805, Precision Scientific Co.).

**Sampling**

Samples for coliform counts were taken from milk before and after addition of *E. coli*, and from curd immediately after cutting and after cooking. The pH was measured on milk before and after addition of *E. coli*, on whey after cutting the curd and after cooking the curd and on curd after the final draining step until the pH reached about 5.6 or until the curd was hooped. Samples of cheese for pH determination and coliform counts were taken on the first 2 d after manufacture, and weekly or biweekly thereafter.

**Enumeration of *E. coli***

The method used was that of Speck et al. (17) to enumerate sublethally injured coliforms. Samples were surface-plated onto Trypticase Soy Agar (TSA, Difco). Plates were held for 1 h at room temperature followed by adding an overlay of Violet Red Bile Agar (VRB, Difco). Plates were then incubated at 37°C for 24 h. This method is referred to as the TSA + VRB surface plating method, and was used to enumerate *E. coli* in all experiments.

Cheese samples were prepared according to specifications in Standard Methods for the Examination of Dairy Products (11). In all instances when enteropathogenic *E. coli* were presumably in the cheese, the "alternative" method was used. This involved weighing a 1 g±10 mg sample of cheese into a sterilized 6-oz Whirl-Pak bag (Nasco), macerating the contents to a fine paste and adding 9 ml of 2% sodium citrate solution at 40°C. Agitation of the bag facilitated production of a fine emulsion which was then plated. In several instances, 11 g of cheese made with *E. coli K-12* and 99 ml of sterile 2% sodium citrate solution were added to a sterile blender, mixed for 2 min, inverted, remixed for 10 sec and then plated (11).

**Measurement of pH, moisture, fat and salt**

The pH of cheese was generally measured (11) with a pH meter.
Complete cover of the bulb and wick of the electrode. About 3 g of cheese was grated into an open plastic petri dish. The grated sample was then tamped into the bottom of a test tube (16 x 150 mm). The electrode was pressed into the test tube until a layer of cheese completely covered both the bulb and wick of the electrode. When a stable pH reading was observed, it was recorded. This was generally about 1 min after the electrode made contact with the cheese sample. However, in studies on cheese made with E. coli K-12 and enteropathogenic E. coli 4608, pH was determined by a quinhydrone technique (14,18), using a saturated calomel half cell, gold electrode and pH meter (Corning, Model 140) set on the millivolt function. Sufficient quinhydrone crystals were mixed with about 3 g of cheese, using a mortar and pestle, to give the cheese a gray color. Cheese was then packed into a tube and the gold electrode inserted into the cheese. The tube and electrode assembly was then placed into a saturated potassium chloride solution as was the reference electrode. The electrical potential was read 90 sec after mixing the cheese and quinhydrone and the reading was converted to pH using a chart. The average of three readings was used as the measure of pH.

The percent of moisture in cheese was determined in triplicate. Moisture contents of 2-3 g of cheeses made with enteropathogenic E. coli were determined by weight difference before and after drying cheese in a vacuum oven for 5 h at 100±2°C, according to Standard Methods (11). The percent of moisture in 2-3 g of cheese made with nonpathogenic E. coli K-12 was determined by weight difference before and after drying in a forced air draft oven for 16 h at 110°C, according to Standard Methods (11). The percent fat was determined in duplicate by the Babcock method (11). The potentiometric method of Dixon (7) was used to determine the percent of salt in the cheese samples. This was done in triplicate.

RESULTS AND DISCUSSION

Growth of E. coli during initial stages of cheese manufacture

In two trials, the population of enterotoxigenic E. coli H10407 and B2C or enteroinvasive 4608 increased 1000-fold before the first draining step of cheesemaking. This occurred 3.5 h after cheesemaking began for E. coli H10407 (Fig. 1), and B2C (Fig. 2), but 3.9 h for E. coli 4608 (Fig. 3). However, enteroinvasive E. coli 0128B12 (data not shown) and nonpathogenic E. coli K-12 (data not shown) only exhibited a 100-fold increase before draining, which occurred 3.5 and 3.8 h after cheesemaking began, respectively. Generation times (Table 2) calculated for the time interval between cutting and draining of curd indicate that E. coli H10407 and 4608 grew faster than did E. coli 0128B12 and B2C in the curd-whey mixture.

Differences in behavior of strains of enteropathogenic E. coli in cheese

Growth and survival of EEC in Colby-like cheese differed among the strains tested. Essentially two types of cheese were made in this project. Those which were Colby-like (Table 1 a, b, c) and those which were of poor quality (Table 1 d, e, f). By law, Colby cheese must have less than 40% moisture and not less than 50% milkfat in the dry matter (3). Cheeses that approached these limits, that were salted when the pH was about 5.6, had a pH of less than 5.4 after pressing overnight and that contained between 1.4 and 2.3% salt (1.7% is recommended) were considered to be Colby-like cheeses. Cheeses not
Enterotoxigenic *E. coli* B2C in cheese of lots #1 and #2 stored at 3±1°C reached peak populations of about $1 \times 10^6/g$ on the first day after manufacture (Fig. 2). This level was reached after overnight pressing in lot #2 and after 1 day of storage at 3±1°C in lot #1. Cheese of lot #1 stored at 3±1°C for about 8 weeks contained virtually no *E. coli* B2C. However, cheese of lot #2 contained approximately $3 \times 10^5 E. coli$ B2C/g after about 12 weeks at 3±1°C. More than 40% (41.6%) moisture in the cheese may partially account for marked survival of these organisms. The pH had remained consistently lower (5.0-5.2) for cheese of lot #1 than for cheese of lot #2 (5.25-5.6) when they were stored at 3±1°C. This may account for the rapid decrease in the population of *E. coli* B2C in cheese of lot #1 stored at 3±1°C. It has been demonstrated (16) that pH values below 5.00 are germicidal to coliforms in cottage cheese. Inactivation and/or inhibition of EEC occurred at pH 5.2-5.5 in brick cheese (7) and at 5.0-5.2 in Camembert cheese (6). Enteroinvasive *E. coli* 4608 reached a maximum population of about $1 \times 10^6/g$ 3.9 h into the cheesemaking process, followed by a gradual decrease in numbers.

The population of *E. coli* 4608, in lots of cheese stored at 3±1°C, approached zero after nearly 4 weeks of storage (Fig. 3). In this instance, both lots of curd were salted at a very low pH (Table 1); however, thereafter pH remained comparable to those of Colby-like cheeses (Fig. 1-3 and Table 1 a-c). In both instances, after overnight pressing, the pH was about 5.3 and increased throughout the storage period to about 5.5. Another factor which may have contributed to the rapid demise of *E. coli* 4608 is the high salt concentration in cheeses of both lots #1 and #2 (2.26 and 2.06%, respectively). For Colby-like cheeses in our studies, survival of enteropathogenic *E. coli* was strain B2C>H10407>4608.

These results are similar to those of Frank et al. (6,7) who found that strain B2C survived longer (6 weeks) than did H10407 (1 week) and 4608 (1 week) in Camembert cheese and in greater numbers (20,000/g) than 4608 (2,000/g) in brick cheese after 7 weeks of storage.

**Survival of strains of EEC at different temperatures**

Storage of cheese made with *E. coli* H10407 or 4608 at 10±1°C apparently had very little effect on survival of these organisms, as compared to their survival at 3±1°C (Fig. 1, 3). However, survival of *E. coli* B2C was greater in both lots of cheese stored at 10±1°C than at 3±1°C (Fig. 2). The pH values of cheese of lot #2 stored at both

![Figure 3. Behavior of enteroinvasive E. coli 4608 and changes in pH during manufacture and storage of cheese.](http://meridian.allenpress.com/jfp/article-pdf/45/4/310/1650799/0362-028x-45_4_310.pdf)

TABLE 2. Generation times of enteropathogenic strains of *E. coli* during the interval between cutting of curd and the end of cook in the manufacture of Colby-like cheese.

<table>
<thead>
<tr>
<th>Strain of <em>E. coli</em></th>
<th>Lot #1</th>
<th>Lot #2</th>
<th>Average ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>H10407 (enterotoxigenic)</td>
<td>19</td>
<td>18</td>
<td>19±1</td>
</tr>
<tr>
<td>B2C (enterotoxigenic)</td>
<td>34</td>
<td>24</td>
<td>29±7</td>
</tr>
<tr>
<td>4608 (enteroinvasive)</td>
<td>18</td>
<td>21</td>
<td>20±2</td>
</tr>
<tr>
<td>0128B12 (enteroinvasive)</td>
<td>24</td>
<td>25</td>
<td>25±1</td>
</tr>
</tbody>
</table>

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temperatures did not differ considerably, hence the difference in survival of \textit{E. coli} B$_2$C in these cheeses may have been a result of the difference in temperature. The difference in population was only about one log cycle after about 12 weeks. No appreciable difference had appeared at about 8 weeks. The difference observed at about 12 weeks may also have been influenced by a non-homogeneous distribution of \textit{E. coli} in the cheese samples that were analyzed. Cheese of lot #1 stored at 3±1°C generally had a lower pH than did cheese of lot #2 stored at 10±1°C. The difference in survival of the bacteria at the two temperatures was greater in lot #1 than in lot #2 (Fig. 2). Doubtless the lower pH of cheese of lot #1 stored at 3±1°C (as opposed to cheese of lot #1 stored at 10±1°C) also played a role in reduction of the population of \textit{E. coli} B$_2$C. \textit{E. coli} B$_2$C survived better overall in cheese of lot #2 than in that of lot #1. This is probably a result of the generally lower pH of cheese of lot #1.

**Survival of EEC in cheese of high moisture and pH**

Two lots of poor quality cheese were made and enteroinvasive \textit{E. coli} 0128B12 served as the test organism. The cheeses were salted at about pH 6.1 and contained about 46 and 45% moisture for lot #1 and lot #2, respectively (Table 1). In both lots of cheese, \textit{E. coli} 0128B12 reached populations in excess of 1 $\times$ 10$^8$/g by the third day after manufacture. These numbers persisted for more than 12 weeks. No appreciable differences in survival were observed at the two storage temperatures or between lots.

**Survival of EEC in cheese of proper pH and moderate moisture**

Cheeses made with enterotoxigenic \textit{E. coli} B$_2$C (Fig. 2) were salted at pH 5.6, reached pH 5.00-5.25 after overnight pressing, and contained about 1.6-1.7% salt (Table 1). However, the moisture content was high (about 41.6%). Numbers of coliforms in these cheeses never rose above 2 $\times$ 10$^6$/g, and generally began to decrease after 1 week of refrigerated storage.

**Influence of salt on survival of EEC**

When cheese was made with \textit{E. coli} H10407 (Fig. 1), lot #2 had 0.5% more salt than did lot #1. The cheeses were very similar in content of moisture and milkfat and in pH of curd at salting (Table 1). No appreciable differences in survival were noted, except that \textit{E. coli} H10407 in cheese of lot #2 (1.92% salt) at 3±1°C persisted at low levels much longer (about 9 weeks) longer than in cheese of lot #1 (1.40% salt) stored at 3±1°C. There is no obvious explanation for this observation.

**Cheese made with nonpathogenic \textit{E. coli} K-12**

Cheeses made with \textit{E. coli} K-12 were unacceptable for human consumption as a result of high pH values (pH at time of salting &gt;6.40), and moderate to high moisture contents (40-48%). In each instance, coliform levels exceeded 1 $\times$ 10$^8$/g shortly after manufacture (Fig. 4 and data not shown). All cheeses containing \textit{E. coli} K-12 were made from milk inoculated to contain about 1000 \textit{E. coli} K-12/g.

**Effect of high pH and moderate moisture content on survival of \textit{E. coli} K-12**

One cheese was made, which contained \textit{E. coli} K-12 and this cheese had a nearly acceptable moisture content (40.36%) but a high pH value [see Table 1, Fig. 4, (lot #2)]. A large number ($\geq$1 $\times$ 10$^8$/g) of coliforms persisted in this cheese for more than 17 weeks. This emphasizes the importance of maintaining a proper pH during cheese manufacture, and suggests that pH may be more important than moisture content in controlling numbers of \textit{E. coli}.

**Influence of salt on survival of \textit{E. coli} K-12 in cheese**

Presence or absence of salt seemed to have very little effect on survival of \textit{E. coli} K-12 during the first 4 weeks of cheese storage (Table 1, Fig. 4 and data not shown). The numbers of \textit{E. coli} K-12 in cheese made with no salt (Fig. 4) or half the normal amount of salt (data not shown) were about 5 $\times$ 10$^8$/g at the fourth week. Numbers of \textit{E. coli} K-12 in cheese made with the full amount of salt were slightly lower (data not shown) at the fourth week. After this point a trend seemed to develop. Numbers of \textit{E. coli} K-12 in saltless cheese and in cheese with about half the proper amount of salt and stored at 3±1°C decreased much slower than they did in cheese stored at 10±1°C. This phenomenon does not appear to be related to pH since the pH of cheese of lot #1 (Fig. 4) stored at 10±1°C was much higher than that of cheese of
lot #1 stored at 3±1°C. One would expect the sample with the higher pH value to have the higher coliform count. This was not true for cheese of lot #1 (Fig. 4). However, when the full amount of salt was added to cheese of high moisture content and pH (data not shown), the trend was diminished if not slightly reversed. It may be that in cheese with low salt there was a greater build-up of toxic metabolic waste products harmful to E. coli as a result of greater metabolic activity at 10±1°C than at 3±1°C. When the full amount of salt was added, the inhibitory effect of salt may have overwhelmed that produced by a higher storage temperature (10±1°C).

**Influence of adding cold water to the curd-whey mixture on survival of E. coli**

During cheesemaking, cold coliform-free tap water was added to the curd-whey mixture after partial draining and the mixture was stirred for different times (Table 1). In general, stirring for a shorter time seemed to result in better acid development and poorer survival of E. coli (Table 1 a-c, Fig. 1-3) than did stirring with cold water for a longer time (Table 1 d-f, Fig. 4, and data not shown). It appears that stirring with cold water for longer than 20 min resulted in cheese with a high pH value, which enhanced survival of E. coli and EEC, thus creating a potential health hazard if the cheese were consumed. The temperature of the curd-whey-water mixture differed only slightly (0.5°C) between cheeses made with EEC and cheeses made with E. coli K-12 (32.8 and 33.3°C, respectively). The same holds true for the temperature of the curds after the water and whey were completely drained. Curd made with E. coli K-12 was also cooked 15 min longer than curd made with EEC and this too may have contributed to the higher pH. These results are reasonable if one considers that lactose is removed from the curd when cold water is added to the system. Lactose is used by the starter culture to produce lactic acid thereby lowering the pH. When the curd-whey mixture is cooked, the curd shrinks and whey is expelled. Some of the lactose is also expelled. Hence longer cooking in addition to longer washing with cold water would contribute to a higher pH. Curds made with EEC B,C and 0128B12 were washed 20 min with cold coliform-free tap water and otherwise treated the same as other curds. There is no obvious reason for the high pH value of the cheese made with EEC 0128B12 and for proper pH development in cheese made with EEC B,C. It should be noted that the decrease in pH of curd containing EEC 4608 before salting was very rapid so that the curd could not be salted at pH 5.6 but rather at 5.2. This curd had been washed the shortest time. Furthermore, cheeses salted or pressed when at a pH above 5.6 were only done so after waiting at least 1 h for the pH to go down after the curds were fully drained.

**DISCUSSION**

Our results indicate that when Colby-like cheese was made from milk contaminated with 100-1000 EEC/ml, the EEC increased 100-1000-fold by the end of the cook, reaching 1 x 10⁶/ml at this time or in 1 to 2 days after the day of manufacture. These numbers began to decrease gradually over a period of weeks as the cheese aged. When cheese was of high pH and moisture content (>40%), the population of EEC and nonpathogenic E. coli continued to increase through the day(s) following manufacture to numbers in excess of 1 x 10⁸/g, which persisted for many weeks. Upon examination after several weeks, these cheeses were softer than those of Colby-like quality, exhibited gassy defects, and some were quite odorous.

It is necessary to ingest about 1 x 10⁸ - 1 x 10¹⁰ enterotoxigenic E. coli or 1 x 10⁶ - 1 x 10⁸ enteroinvasive E. coli to become ill (2). If one assumes that an average person consumes about 200 g of cheese at one time, then about 5 x 10³ to 5 x 10⁵ enteroinvasive E. coli/g of cheese or 5 x 10⁴ to 5 x 10⁶ enterotoxigenic EEC/g of cheese are required to make one ill.

Furthermore, the law requires (3) that Colby cheese made from raw milk must be stored at not less than 35°F (1.7°C) for no less than 60 days before releasing it to consumer channels. Numbers of EEC in the cheeses of Colby-like composition (Fig. 1-3) were below the levels indicated in the previous paragraph after 60 days of storage. However, if the cheeses of high pH (salted at about pH 6.1 and having a pH of 5.8 and 6.0 at one day and moisture (about 45-46%) made with E. coli 0128B12 were released to consumer channels after even 86 days of refrigerated storage, they would have constituted a potential health problem. If the cheeses of high moisture content and pH made with E. coli K-12 contained EEC instead of nonpathogenic E. coli K-12, they would still have constituted a potential health hazard if released after 90 days of refrigerated storage.

A characteristic step in the manufacture of Colby cheese is the addition of cold coliform-free tap water to the partially drained curd-whey mixture. This is done to facilitate removal of lactose and to provide a more open structure to the cheese. Lactose is metabolized by the lactic starter culture with the resultant production of lactic acid and a reduction in pH. Hence, addition of water to the curd-whey mixture is crucial in the pH control of Colby cheese. Done properly this step should result in the milder cheese we know as Colby. However, if this process continues to long (>20 min in this study) a cheese of high pH may result, which will permit growth (during initial cheesemaking) and subsequent survival of large numbers of E. coli (Table 1, Fig. 4, and data not shown). Such cheese would be potentially hazardous if any EEC were present.

It was suggested by Frank et al. (7) that enterotoxigenic E. coli may produce enterotoxins during growth, which would be left behind in the cheese after the numbers of enterotoxigenic EEC had decreased. Conceivably this cheese, if consumed, could cause gastrointestinal illness. No assays were done on cheese made with enterotoxigenic EEC for presence of pre-formed enterotoxins. However, Lovett et al. (10) stated that presence of preformed E. coli...
enterotoxins in protein foods is unlikely because the foods must be maintained at the optimum temperature (35°C) for enterotoxin production for at least 24 h. Any deviation from this optimum delays the appearance of enterotoxin.

EEC may persist in Colby-like cheese at low levels (<350/g) for many weeks (Fig. 1). This may present a potential problem if temperature abuse should occur, or if the cheese becomes an ingredient of a product that would permit growth of EEC. Cheese made with the enterotoxic EEC B2C had a moisture content 1.6% in excess of the legal limit shortly after manufacture. In lot #2 of this cheese stored at 3±1°C, E. coli B2C was still present at about 1 x 10^4/g [2.2°C - 4.4°C for 2-3 months storage is recommended for Colby cheese (9)]. After 86 days of storage, E. coli B2C was still present in excess of 1 x 10^3/g. Temperature-abuse might have resulted in a potentially harmful product had it been released after 90 days of storage. If this organism (E. coli B2C) were enteroinvasive, this cheese (lot #2 at 3±1°C) would have presented a potential health hazard if released into consumer channels after 60 days of storage. The amount of added salt, the difference in strain survival between refrigeration at 3±1°C and unrefrigerated storage, and the moisture content of the cheese appeared to play a lesser role in survival of E. coli in Colby-like cheese than did pH control.

Frank et al. (6, 7) studied survival of EEC in Camembert and brick cheese. When one compares their results to ours, it appears that Camembert cheese was more inhibitory to EEC than was Colby-like or brick cheese. For instance, when E. coli B2C was added to milk at about 100/ml, it survived better in Colby-like cheese (see Fig. 2) than in Camembert cheese (6) in which it was not detectable after 6 weeks of storage. Numbers of E. coli B2C in Colby-like cheese after 7 weeks were comparable to those in brick cheese (7) at the same time (about 20,000/g). Frank et al. (7) also made brick cheese from pasteurized whole milk containing E. coli 4608 at a concentration of 500/ml. We made some Colby-like cheese from pasteurized whole milk containing 1000 E. coli 4608/ml. E. coli 4608 survived 4 weeks in our cheese and was present in brick cheese at 2000/g after 7 weeks. Frank et al. (6) suggested that the lower pH of unripened Camembert cheese accounted for the lower survival of EEC in Camembert cheese than in brick cheese. This is also likely to be true for Colby-like cheese.

A recent survey of different types of cheese (8) in Ames, Iowa, showed that intermediate-moisture cheeses contained E. coli (41% of intermediate-moisture cheeses contained E. coli) more often than did cheese in any other category. These results suggested that intermediate-moisture cheeses like Colby could be potential vehicles for transmission of EEC strains. Results of this study indicate that this can be true, particularly when the cheese is made improperly.

Our data emphasize the importance of pH regulation, proper sanitation and use of proper procedures in the manufacture of Colby cheese. The same, of course, also applies to other varieties of cheese.

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