UNIVERSITY OF IDAHO
College of Agriculture

Suggestions for Preventing Cheese Starter Failures

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Department of Dairy Husbandry

IDAHO Agricultural Experiment Station

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Follow These Steps

1. Initiate a testing program for growth-inhibiting substances in each patron's milk.
2. Use non-fat dry milk known to be free of growth-inhibiting substances for the preparation of mother and bulk cultures.
3. Initiate a testing procedure for the detection of bacteriophage infections.
4. Arrange the cheese plant to provide for:
   (a) a separate starter preparation room.
   (b) a separate whey separation room.
   Use air-tight separators.
5. Use the utmost care in the preparation of the mother and bulk cultures.
6. Do not use cooking temperatures in excess of 102° F. in the making of cheddar or other varieties of cheese that repuire continued acid production after cooking.
7. At regular intervals, check the accuracy of thermometers used in making cheese.

Suggestions for Preventing Cheese Starter Failures

J. C. BOYD and R. A. HIBBS*

Importance to the Dairy Industry of Idaho

The failure of bacterial cultures to show normal growth and acid production causes a condition generally referred to in the cheese industry as "starter failure". Because a considerable portion of the milk supply in Idaho, as well as in other states, is manufactured into various types of cheese, this problem has real economic significance.

During the last few years, "starter failure" has become more noticeable in Idaho, as well as in other major cheese-producing areas. This is evident from contacts with the cheese industry and the fact that the Idaho Milk Processors Association and the Idaho Dairymen's Association passed resolutions at their meetings in 1953 requesting the University of Idaho to assist in this problem.

Some Common Causes of Starter Trouble

In recent years, slow starters or complete starter failures have been largely attributed to the presence of growth-inhibiting substances; that is, antibiotics in the milk supply. It should be recognized, however, that poor starter-carrying procedures often result in contamination of the starter with undesirable organisms which cause starter failures. The possibility of bacteriophage infections in the cheese plant should also be recognized as a cause of starter failures. The term bacteriophage comes from the words "bacterium" and "phagein", which means to eat. The term then means an agent (virus) that eats bacteria. Cheese cooking temperatures above 102° F. also may inactivate many of the acid-producing organisms and be the cause of slow acid production during the cheesemaking process

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The Problem of Inhibitory Substances in the Milk Supply

Substances which will inhibit the growth of lactic acid starter organisms in milk may be classified into three groups: (1)antibiotics; that is, penicillin, streptomycin, terramycin, sulpha drugs, etc.; (2) sterilizing agents, such as quaternary ammonium compounds (usually chlorine compounds are not a factor); and (3) inhibiting or antibiotic-like substances produced by bacterial growth in the milk.

The presence of residual amounts of antibiotics in the milk supply comes about as the result of treating dairy cows for mastitis with antibiotics, and the failure of the dairy farmer to withold the milk from the treated quarters from the cheese milk supply. Research has shown (1) that milk from quarters treated with antibiotics should be witheld for 3 days (six milkings) after last treatment takes place, but (2) that the milk from untreated quarters of the same cow is apparently normal. (Hansen, *et al.*, 1951). The effect of intra-muscular or intra-veinous injections of antibiotics for other types of infection; i. e., foot rot, etc., on the milk is not clear as published reports are contradictory. Any animal under any kind of antibiotic treatment would be classified as a diseased animal, however, and the sale of milk from a diseased animal is prohibited by law.

The presence of small amounts of quaternary ammonium compounds in milk will also cause slow acid production or complete starter failure (Miller and Elliker-1951, and Curry and Barber-1952). Small amounts of chlorine sterilizing compounds do not have this property, however, as they are quickly inactivated by milk or other organic matter and for this reason are preferred for sterilizing cheesemaking equipment. Five to 30 p.p.m. of quaternary ammonium compounds are necessary to cause serious starter troubles. Usually when these amounts are present in the milk supply, the compounds have been: (1) deliberately misused and added to the milk for the purpose of preserving it, or (2) by accident milk has been poured into a can or tank containing a solution of quaternary ammonium.

There have been research reports which show that the growth of certain bacteria in milk may produce substances which are inhibitory to lactic acid starter organisms (Auclair 1954). This condition may exist when milk is used which contains an excessively large number of bacteria. Milk from cows infected with mastitis apparently contains some inhibitory substances also. (Babel 1952)

The Extent of Inhibiting Substances in Idaho's Milk

During a 1954 survey, 1,146 samples of different lots of Idaho

milk were analyzed for the presence of inhibitory substances. These samples were collected in the Moscow, Lewiston, Boise and Twin Falls areas. Five and one-tenth (5.1) percent of these lots of milk would not support bacterial growth when lactic acid starters were added at the rate of 1 percent and the samples were incubated at 72° F. for 14 to 16 hours. Figure 1 shows the distribution of the samples, which contained growth-inhibiting substances according to the volume of milk each sample represented. These results indicate that 57.8 percent of the samples that would not support bacterial growth came from lots of milk which contained 200 gallons or less. Only 1.8 percent of

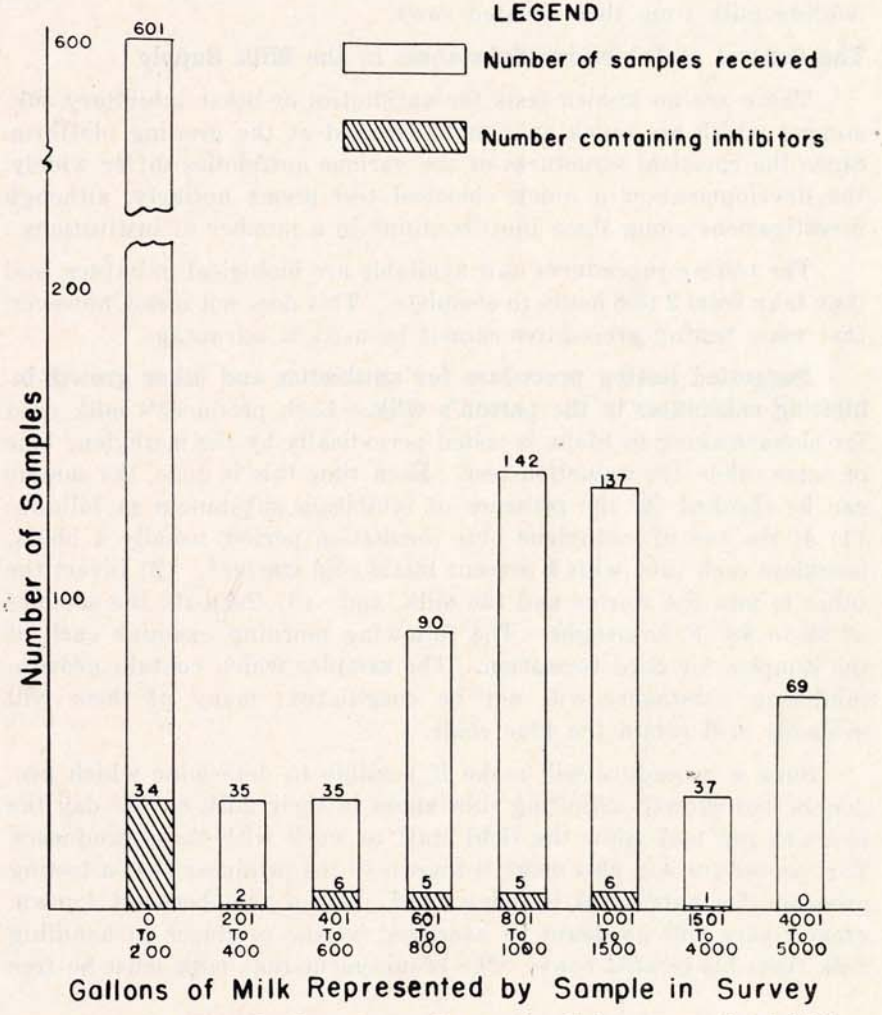

Figure 1.--Distribution of samples analyzed in survey for inhibitory material.

the samples containing growth-inhibiting substances were found in lots of milk which contained 1,500 to 4,000 gallons and no samples containing inhibiting substances were found in the 69 lots of milk, each containing over 4,000 gallons of milk, These results show that it is advantageous to combine milk from a large number of producers.

The percentage of milk lots that contained growth-inhibiting substances varied considerably between sections of the state and also between dairy plants in the same locality. If it is assumed that the inhibition was caused by the presence of antibiotics in the milk, then the results indicate a considerable difference in the cooperation being received from dairy farmers in different localities relative to withholding milk from their treated cows.

The Control of Inhibitory Substances in the Milk Supply

There are no known tests for antibiotics or other inhibitory substances which are quick enough to be used at the grading platform. Since the chemical structures of the various antibiotics differ widely, the development of a quick chemical test seems unlikely, although investigations along these lines continue in a number of institutions.

The testing procedures now available are biological in nature, and they take from 2 to 6 hours to complete. This does not mean, however, that some testing procedures cannot be used to advantage.

Suggested testing procedure for antibiotics and other growth-inhibiting substances in the patron's milk.—Each producer's milk used for cheesemaking in Idaho is tested periodically by the methylene blue or some other dye reduction test. Each time this is done, the sample can be checked for the presence of inhibiting substances as follows: (1) at the end of methylene blue incubation period, usually 4 hours, inoculate each tube with 1 percent lactic acid starter*, (2) invert the tubes to mix the starter and the milk, and (3) incubate the samples at 95 to 98° F. overnight. The following morning examine each of the samples for curd formation. The samples which contain growth-inhibiting substances will not be coagulated; many of them will probably still retain the blue color.

Such a procedure will make it possible to determine which producers had growth-inhibiting substances in their milk on the day the test was run and allow the field staff to work with those producers. This procedure will also make it known to the producer that a testing program for antibiotics is being used. When this becomes known, greater care will no doubt be exercised by the producer in handling milk from his treated cows. The requirement that milk must be free

*To facilitiate measuring the starter, add 11 ml of starter to 99 ml of sterile milk, shake well, and add 1 ml of the mixture to each methylene blue tube.

of any antibiotics might well be a part of the premium milk program† now in effect in most of Idaho's cheese-manufacturing areas.

This testing procedure will not identify the inhibiting substance, but more than likely it would be found upon investigation that the inhibition was due to the presence of antibiotics or quaternary ammonium compounds in the milk.

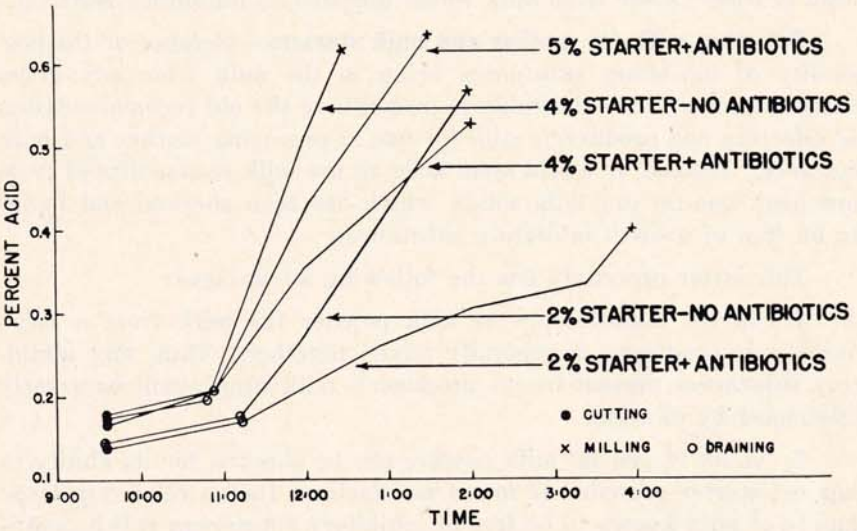

Figure 2.--The effect of increasing amounts of starter when milk contains antibiotics.

Testing bulk milk for antibiotics and other growth-inhibiting substances.—In cases where plant storage facilities allow milk to be held overnight, these lots of milk can be tested for growth-inhibiting substances by the same procedure as outlined above; that is, by inoculating a sample with 1 percent starter and incubating it overnight. Failure of these samples to set a firm curd and develop a normal acidity (0.7 to 0.8 percent) will indicate the presence of inhibiting substances.

In cases where this type of testing procedure is used, a control milk sample should be set up. A sample of re-constituted, non-fat dry-milk solids, containing 9.5 to 10 percent total solids, which has been previously tested and found to support good starter activity, may be used for such a control. Samples of milk which, when inoculated with the same starter, produce 25 percent less acid than the control can be considered to contain some inhibiting substances. For example: if the control tube produces 0.75 percent acidity, then any

†This program provides a premium payment for milk that is mechanically refrigerated, and meets certain methylene blue and sediment requirements.

sample that did not produce 0.56 percent acid [.75 - (.75 x 0.25) = .5625] may be considered to contain some inhibitory substances.

In cases where only slight inhibition of the cheese starter bacteria takes place, experiments have shown that normal acid production might be obtained by increasing the rate of innoculation with starter to 4 or 5 percent (see Figure 2). The quality of the finished cheese made under these conditions was found to be reasonably acceptable, but never as high as cheese made from milk which contains no inhibitory material.

Selecting milk for mother and bulk starters.—Because of the possibility of inhibitory substances being in the milk from any given dairy farmer it seems desirable to discontinue the old recommendation of selecting one producer's milk for use in preparing mother and bulk cultures. Instead, it would seem safer to use milk re-constituted from low heat, non-fat dry milk solids, which has been checked and found to be free of growth inhibiting substances.

This latter procedure has the following advantages:

1. In the manufacture of milk powder the milk from a large number of producers is generally mixed together. Thus, any inhibitory substances present in one producer's milk supply will be greatly minimized by dilution.

2. A lot of non-fat milk powder can be checked for its ability to support starter growth. If found satisfactory, then a relatively large supply of milk known to be free of inhibitory substances will be available for starter making. The milk supply can thus be ruled out as a cause of starter failures. Sometimes non-fat dry milk powder is used to standardize cheese milk. In such cases each lot of powder should be checked for its ability to support bacterial growth before being used.

Care should be exercised in making up the reconstituted milk to be sure that the concentration in as uniform as possible from day to day. A reconstituted milk containing 10 percent total solids has been found to be satisfactory. This may be prepared by adding 10 grams of milk powder to 90 ml of water and stirring or shaking it until all the milk powder is disolved.

The Problem of Bacteriophage

What Is Bacteriophage

The term bacteriophage has already been defined as an agent (virus) that eats bacteria. The bacteriophage particles look like tadpoles when seen with an electron microscope. Figure 3 shows the relative size of bacteria cells and bacteriophage particles.

Bacteriophage particles are more or less specific, which means that they generally attack one strain of bacteria, but do not attack another (Hammer 1948). They are more resistant to heat than many bacteria and may survive ordinary pasteurization temperatures. Nine bacteriophage strains were found to resist heat treatment from 158 to 167° F. for 30 minutes (Whitehead and Hunter 1939). Bacteriophage survive drying and these particles have been known to live 72 months in a dry state (Prouty 1953). They are very small and easily carried by air currents; therefore, dust and air-borne contamination is to be guarded against. The multiplication of bacteriophage requires the presence of a host bacterial cell susceptable to the bacteriophage.

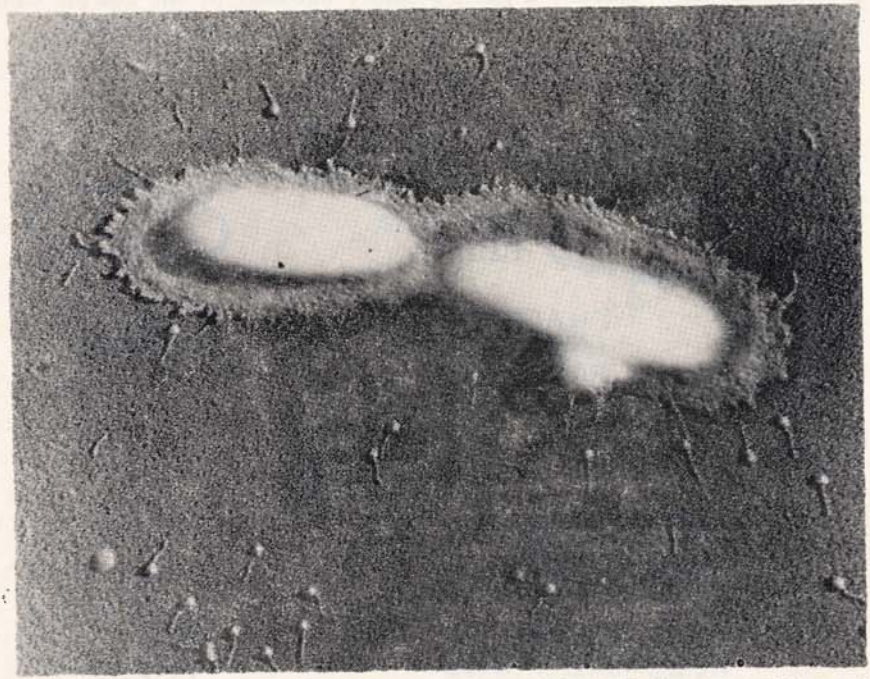

Figure 3.--The relative size and shape of bacteriophage particles and the bacteria S. lactis. **(Courtesy Iowa Agricultural Experiment Station and the Journal of Bacteriology).**

Some Symptons of Bacteriophage Infections in the Cheese Factory

The most common result of bacteriophage infections is a slow acid production in case of moderate infection and complete failure of acid production where the infection is heavy. The degree to which the acid production will be inhibited depends upon the percentage of bacteria destroyed. Figure 4 shows how bacteriophage attack a bacterial cell, multiply in it and eventually destroy it. Table 1 shows the effect of bacteriophage infections on acid production during the manufacture

of cheddar cheese and Table 2 shows their destruction of bacterial cells during the manufacture of cottage cheese. It will be noted in table 1 that normal acid production took place for about the first 2 hours, then ceased. Also in Table 2, almost normal bacterial growth took place the first hour, but then destruction of the bacterial cells was rapid until at the end of 3 hours only 62 bacteria per ml. were present in the milk contaminated with bacteriophage.

In case of a starter of a single strain of bacteria, all the cells may be destroyed and the result will be no acid production. In case of starters of mixed strain, one or more strains may be destroyed, but the strains remaining will grow. However, acid production will probably be slow because a part, possibly one-half of the starter has been destroyed.

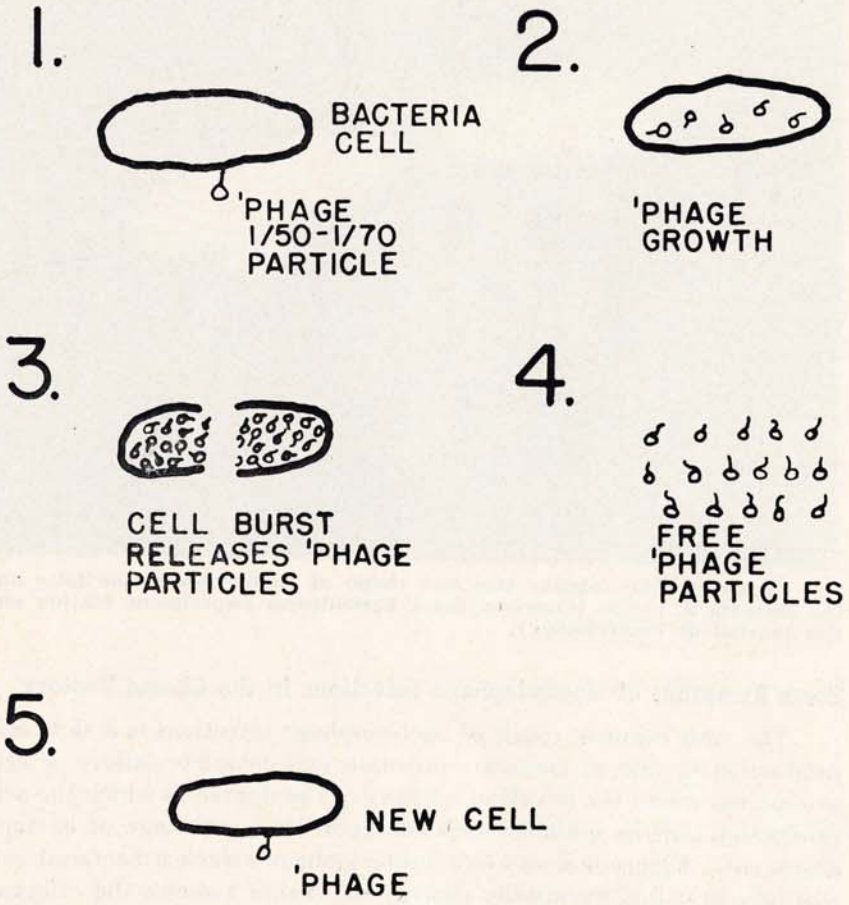

Figure 4.--How bacteriophage particles multiply.

Table 1.—Action of bacteriophage on a cheese culture during the manufacture of cheddar cheese

	No bacteriophage		Bacteriophage present	
Step in manufacture	Time	Titrable acidity (per cent)	Time	Titrable acidity (per cent)
Milk (past.)	9:00	0.17	9:00	0.17
Culture added (1%)	9:15	0.18	9:15	0.18
Setting	10:15	0.185	10:15	0.185
Cutting	10:45	0.13	10:45	0.12
Draining	12:15	0.15	12:30	0.14
Milling	2:45	0.51	4:30	0.18

—1.—Sta. Bul. 573, Purdue University.

Table 2.—Action of bacteriophage on a cheese during the manufacture of cottage cheese by the short time method.

Inoculation Time after (hrs.)	Bacteria count control vat (per ml)	Bacteria count contaminated vat (per ml)	Bacteriophage concentration in contaminated vat (per ml)
0	12,300,000	14,700,000	10,000
1	16,700,000	15.100,000	1,000,000
2	38,000,000	240,000	1,000,000,000
3	98,000,000	62	"
4	350,000,000	79	"
5	750,000,000	240	"

—1.—Sta. Bul. 573, Purdue University.

Infected mother and bulk cultures may appear slow or even normal, but contain large numbers of bacteriophage which continue to act in the cheese vat and prevent acid production. **Infected bulk cultures are considered to be a major source of bacteriophage trouble** (Matteck *et al.* 1944).

In cases where no acid is produced the loss is obvious. In cases where the acid production is slow, the moisture control of the cheese will be difficult. Extra time will be required to complete the manufacturing process and the quality of cured cheese probably will be poor.

The Extent of Bacteriophage Infection in Idaho

For several months during 1954, the Department of Dairy Husbandry invited cheese makers to send in samples of cheese whey and starter from each vat of cheese in which acid production was slow or a complete failure. Samples were received from every major cheese-producing section of the state. Approximately 78 percent of these samples have shown the presence of bacteriophage. Thus, bacteriophage infections appear to be an important factor in Idaho's cheese industry.

Detection of Bacteriophage

The presence of bacteriophage can be demonstrated by the following procedure.

1. Obtain some whey from the suspected cheese vat or bulk culture.

2. Prepare three flasks of sterilized milk, 100 ml. each, known to be free of growth-inhibiting substances. Freedom from growth-inhibiting substances can be determined by the procedure outlined on page 7.

3. Inoculate one flask with the **same mother starter** or bulk culture used in the suspected cheese vat, using a 1 percent inoculation.

4. Inoculate the second flask with 1 percent of the **same starter** used in the suspected cheese vat, but to this flask add 2 or 3 ml. of whey from the suspected cheese vat.

5. Inoculate the third flask with 1 percent of the **same starter** and add 2 to 3 ml. of whey from the suspected cheese vat, which has been heated to 180° F. for 10 minutes and then cooled.

6. Incubate all samples 6 hours at 86 to 90° F. and determine the acid production by titrating with N/10 sodium hydroxide.

Flask 1 is the control and should produce a normal acidity. If bacteriophage is present in the whey, flask 2 will not produce as much acid, possibly none at all; but flask 3 should produce an acidity comparable to flask 1.

The explanation is that in flask 2 the organisms will be destroyed by the bacteriophage which is added in the raw cheese whey. In flask 3, however, the phage particles will be destroyed by heat and normal acid production should take place.

Suggestions for Control of Bacteriophage in the Cheese Factory

Starter preparation room.—Practically every report published on the subject of bacteriophage points to the desirability of having a

starter preparation room separate from the cheesemaking and whey separation room. Some authors suggest separate buildings for starter preparation.

A starter preparation room should be small, have walls and ceilings of smooth washable material, and contain no pipes or ledges where dust may collect. It would be desirable, if possible, to have the room under forced ventilation with filtered air. It should be located away from the whey storage and separation area preferably on the windward side of the building.

The starter room should be equipped with a hot water bath or pressure cooker for sterilizing milk and equipment, containers for the mother cultures so designed that the mouths are relatively small, pipettes or glass tubes for transferring the cultures, a bunsen burner or some other gas flame, and a constant-temperature incubator.

A laboratory coat should be available at the door and be put on each time someone enters the room. Hand-washing facilities should be near the door; and no one should enter the room from the cheesemaking, cheese curing, or whey separation areas without washing their hands. The floor of the starter room should be scrubbed and sterilized each day. No equipment should be brought from the cheese room or whey separating room into the starter room without first sterilizing it. This includes the 10-gallon cans used to transfer the starter to the cheesemaking room. Prior to bringing cans into the starter room, they should be submerged in a 200 p.p.m. chlorine solution.

In preparing mother cultures, do not make a transfer without first flaming both the flask you are transferring from and the one you are transferring to. The use of the pipettes in making the transfer is to be desired over the practice of pouring from one bottle to the other.

The use of cotton plugs in the mother culture bottles is suggested. Papers should also be used over the cotton plugs. The cotton plugs will prevent contamination from the air drawn into the bottle as it cools after sterilization. The bottles should not be filled more than half full and care should be taken not to get the cotton plugs wet. Use a new cotton plug each time. All equipment and milk should be sterilized at 185 to 190° F. for 1 hour or heated under 15 lbs. steam pressure for 15 minutes.

In preparing bulk cultures it would be preferable to have the bulk culture tank in the starter room. Heat the milk to 185 to 190° F. for 1 hour. Before cooling starts, spray the room with a fine mist of chlorine solution (500 p.p.m. of available chlorine) and keep the room closed

until cooling is accomplished. This will kill air-borne contamination that might be drawn into the tank as the milk is cooled after sterilizing.

Just prior to making transfers, again spray the starter room with chlorine solution and leave closed for 10 to 20 minutes.

If starter room facilities as described above are not possible, then the use of 10-gallon cans, each containing about 8 gallons of milk, is suggested rather than a bulk starter tank. The 10-gallon cans will have less milk exposed to the air than a bulkstarter tank.

In any event, never place a thermometer, stirring rod, or any other instrument that has been in a cheese vat into the starter tank without first washing and sterilizing it. Do not open the lid of the starter tank any more than necessary to make the inoculation.

Whey storage and separating facilities.—The facilities for whey storage and separation should be in a separate room from the cheesemaking room and as far away from the starter room as possible, preferably on the down wind side of the building. **Infected whey is one of the major sources of bacteriophage trouble.** The separation of whey with an open-spout separator produces a fine mist that is carried on air currents for considerable distances. The separator room need not be big. It would be desirable to have this room separately ventilated, possibly with an exhaust fan separate from the rest of the factory. After the clean-up at the end of the day, fogging of the room with a chlorine solution (500 p.p.m. available chlorine) would be desirable.

Whey storage facilities should be cleaned periodically and sterilized inside and out with a chlorine solution.

The cheesemaking room.—The room should be constructed so there is a minimum of places for dust to collect.

In sanitizing the cheesemaking equipment, the use of chlorine sprays (500 p.p.m.), directed onto the surfaces of cheese vats, presses, and other equipment that cannot be completely submerged or flooded is suggested. The use of chlorine-spraying equipment will assure a better coverage and penetration into cracks than spreading the chlorine solution over the surfaces with a brush. Cheese rakes, shovels, and all other equipment should be submerged in a chlorine solution for at least 2 minutes before used. No wooden rakes or other equipment should be used.

When it is necessary to use a cheese vat more than once a day, the vat should be thoroughly cleaned and sterilized before the milk for the second run is placed in it. A slight contamination in the first lot of milk may be disastrous to cultures used in the second lot.

The use of chemical sterilizers other than chlorine compounds.—When chlorine compounds are used as sprays or to fog a room, they settle out in time onto the equipment. Because it is not practical to rinse the equipment off after fogging a room and because of the corrosive action of chlorine, its use for this purpose is considered to be somewhat undesirable. As a result, several investigators have searched for other agents which would be effective in destroying bacteriophage, but less corrosive.

Quaternary ammonium compounds have been studied and have been found effective when used to rinse cheesemaking equipment (Prouty 1949 and Bennett and Nelson 1954a). However, when used as fine mist to fog the air in a room, Bennett and Nelson 1954 found quaternary ammonium compounds to be ineffective. Also, there is the danger of contaminating the milk supply with these materials, which in themselves will cause slow acid production or complete starter failures.

The use of glycols for spraying rooms for the destruction of bacteriophage has also been studied, but has been found to be ineffective by at least two separate investigators (Wolf *et al.*, 1946 and Bennett and Nelson 1954).

Thus, recognizing that hypochlorite solutions are somewhat corrosive and irritating to plant personnel, they appear to be the best material presently available for spraying or fogging a room for the purpose of destroying air born bacteriophage particles.

The selection and use of starters.—Because bacteriophages are fairly specific for given strains of bacteria, there are advantages to changing the source of the starter at regular intervals.

It would be advantageous, for example, to carry four starters each from a different source, and to use a different one each day until all four have been used. On the fifth day starter number one could be used again.

Assuming that bacteriophage infection develops against one starter, the above procedure has the advantage, then, of having 4 days of clean-up and sanitizing to kill these particular bacteriophage particles which are attacking a given starter before that starter is used again in cheesemaking. As this bacteriophage will probably affect only the one culture, the remaining three cultures can probably be used without trouble and the bacteriophage should be destroyed before the susceptable culture is again used.

Some do's and don'ts in cheese plant arrangement.—Figure 5 shows a desirable arrangement of a cheese factory. The starter room is partitioned off; also, the receiving room, and whey separation and storage areas are partioned off from the cheesemaking room. An exhaust fan is located in the whey separation room. Notice there is no opening from the starter room directly into the cheesemaking or curing room. Also, this room is located up wind from the whey separation and storage area.

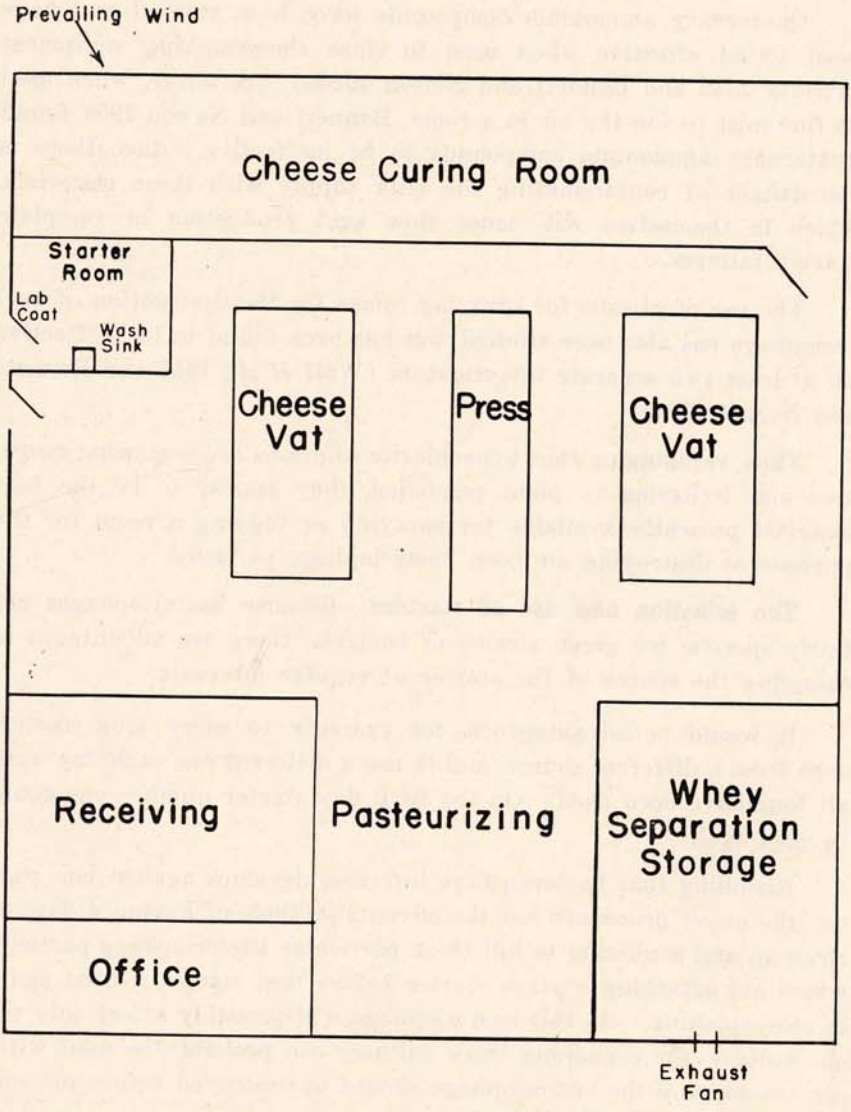

Figure 5.--Desirable arrangement of a cheese factory.

Figure 6 shows some undesirable features of a cheese factory arrangement. All the operations are carried on in one room. There is no starter preparation room and the ventilator is so located that air currents are drawn from the whey separation area over the entire cheesemaking room area. This allows whey infected with bacteriophage particles to be dispersed into the air from the mist of the separator and to be carried on air currents to practically every point in the plant.

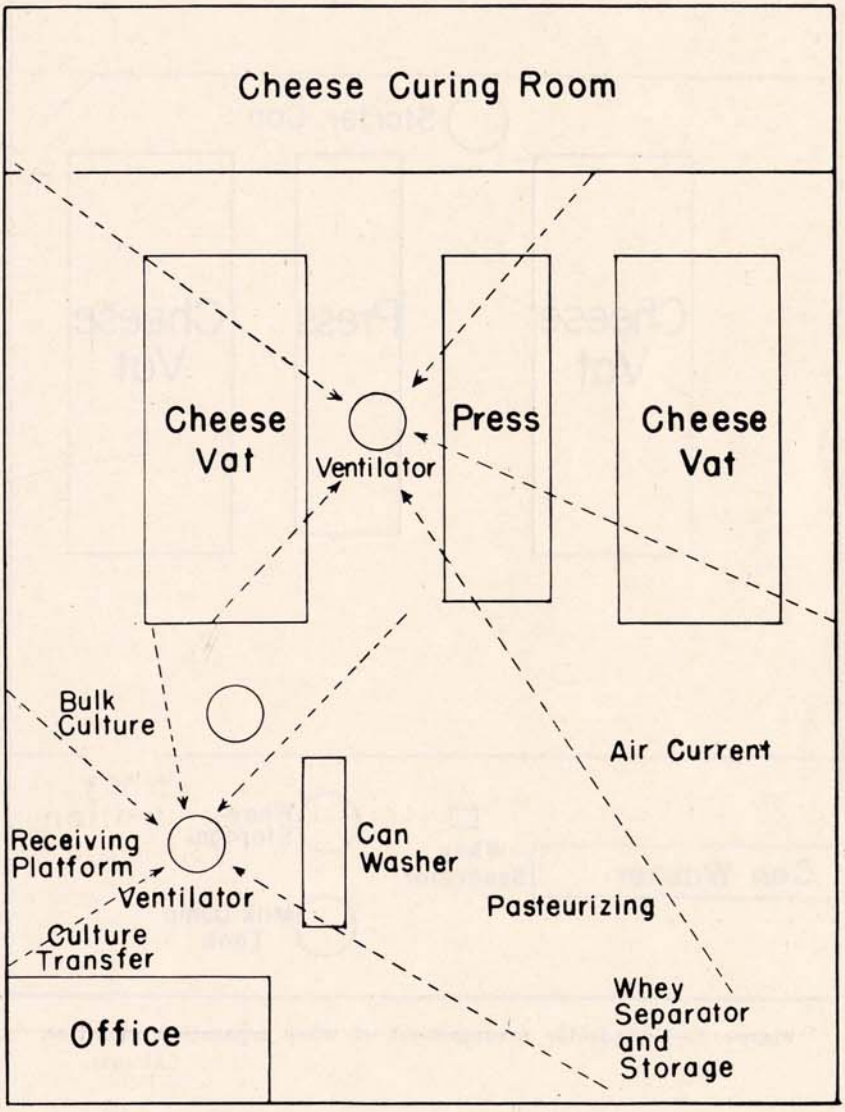

Figure 6.--Undesirable arrangement of a cheese factory.

Figure 7 shows the whey separation facilities in the receiving room. This allows the whey mist from the separator to contaminate all receiving equipment and be carried into the milk supply. Bacteriophage particles are seldom killed by ordinary pasteurization.

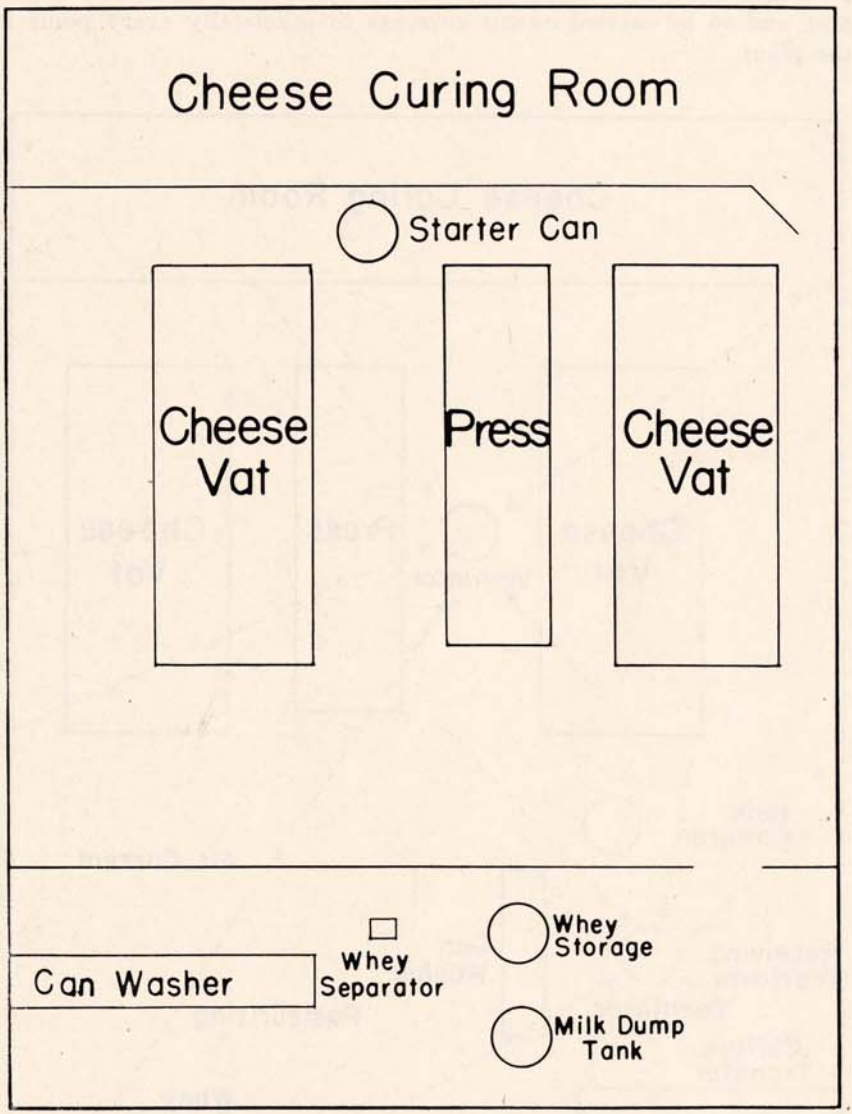

Figure 7.--Undesirable arrangement of whey separation facilities.

The Influence of Cooking Temperatures on Acid Protection

Most cheese cultures produce acid rapidly at temperatures ranging from 70 to 102° F. Temperatures above 102° F. may result in a pronounced decrease in acid production (Babel 1952). The use of temperatures above 102° F. as a means of speeding up the expulsion of whey may result in slow acid production after the whey is drained. Usually it is after the whey is drained that the action of bacteriophage is first evident, and thus, it may be difficult to determine whether slow acid production is due to the cooking temperature or the action of bacteriophage.

Dairy thermometers have been found to read as much as 4° F. below the actual temperature. The use of such thermometers will result in slow acid production. In cheese, such as cheddar, which requires continued acid production after cooking, a lower cooking temperature and a somewhat longer holding period to firm the curd may result in a shorter over-all manufacturing time, because of faster acid production after draining the whey. **Dairy thermometers used in cheese factories should be checked frequently for accuracy.**

Acknowledgments

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Idaho

Boise Health Department, Boise
Cream Spot Dairy, Boise
Gem Valley Swiss Cheese Factory, Grace
Grays Lake Cheese Company, Wayan
Home Dairies, Nampa
Idaho Dairy Products, Moscow
Jerome Cooperative Creamery, Twin Falls
Lewiston Health Department, Lewiston
Nelson-Ricks Creamery Company, Rexburg
Sanitary Dairy, Moscow
Smith Dairy Products, Buhl
Upper Snake River Valley Dairymen's Association, Rexburg
Van's Creamery, Coeur d' Alene
Young's Dairy Products Company, Twin Falls

Washington

Milky Way Dairy, Pullman

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