Microorganisms [illegible] with all of the food we eat. They may influence the quality, availability and quantity of our food. [illegible] with a variety of microorganisms. The magnitude of this microbial contamination reflects one or more of the following: the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage. It is desirable to maintain a very low microbial level of contamination on raw foods; the presence of extremely large numbers of microorganisms suggests that some undesirable events have occurred and that the food is indeed susceptible to further deterioration.

Meats

The carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only nominal surface contamination while the inner tissues are sterile. Fresh meat cut from the chilled carcass has its surface contaminated with microorganisms characteristic of the environment and the implements (saws or knives) used to cut the meat. Each new surface of meat, resulting from a new cut, adds more microorganisms to the exposed tissue. The ultimate in providing new surfaces and potential contamination of meat surfaces occurs in the process of making hamburger.

To improve the microbiological quality of meats, particularly ground beef (in addition to cold cuts and frankfurters), most states have adopted standards, or are considering establishing regulations, to require microbiological standards for these products at the time of purchase.

Among the more common species of bacteria occurring on fresh meats are pseudomonads, staphylococci, micrococci, enterococci, and coliforms. The low temperature at which fresh meats are held favors the growth of psychrophilic microorganisms.

Poultry

Freshly dressed eviscerated poultry have a bacterial flora on their surface (skin) that originates from the bacteria normally present on the live birds and from the manipulations during killing, defeathering, and evisceration. Under good sanitary conditions the bacterial count has been reported to be from 100 to 1,000 bacteria per square centimeter of skin surface, whereas under less sanitary conditions the count may increase 100-fold or more. Pseudomonads constitute the major contaminants on the skin of freshly dressed poultry.

Eggs

The interior of a freshly laid egg is usually free of microorganisms; its subsequent microbial content is determined by the sanitary conditions under which it is held, as well as the conditions of storage, i.e., temperature and humidity. Microorganisms, particularly bacteria and molds, may enter the egg through cracks in the shells or penetrate the shells when the "bloom" (thin protein coat) covering the shell deteriorates. The types of microorganisms involved reflect those present in the environment.

Fruits and Vegetables

Fruits and vegetables are normally susceptible to infection by bacteria, fungi, and viruses. Microbial invasion of plant tissue can occur during various stages of fruit and vegetable development, and, hence, to the extent that the tissues are invaded, the likelihood of spoilage is increased. A second factor contributing to the microbial contamination of fruits and vegetables pertains to their postharvest handling. Mechanical handling is likely to produce breaks in the tissue which facilitates invasion by microorganisms. The pH of fruits is relatively acid, ranging from 2.3 for lemons to 5.0 for bananas. This restricts bacterial growth but does not retard fungal growth. The pH range for vegetables is slightly higher, pH 5.0 to 7.0, and hence they are more susceptible than fruits to attack by bacteria.

Shellfish and Finfish

The microbial flora of freshly caught oysters, clams, fish, and other aquatic specimens is very largely a reflection of the microbial quality of the waters where they are harvested. Of particular significance is whether the water is sewage-polluted, in which case the seafood is potentially capable of transmitting various pathogenic microorganisms. The marine bacterium *Vibrio parahaemolyticus* has been responsible for a number of gastroenteritis epidemics in the United States due to consumption of raw or inadequately cooked seafood. This organism occurs widely in the Atlantic, Pacific, and Gulf Coast waters and has been isolated from seafood samples including fish, shellfish, and crustaceans. Shellfish that grow in contaminated water can concentrate viruses and may be sources of hepatitis infection. For example, raw oysters and clams from polluted waters have caused numerous epidemics in various parts of the world.

Milk

At the time it is drawn from the udder of a healthy animal, milk contains organisms that have entered the teat canal through the teat opening. They are mechanically flushed out during milking. The number present at the time of milking has been reported to range between several hundred and several thousand per milliliter. The counts vary among cows and among the quarters of the same cow and are highest during the initial stages of milking. From the time the milk leaves the udder until it is dispensed into containers, everything with which it comes into contact is a potential source of more microorganisms. This includes the air in the environment, the milking equipment, and the personnel. Disregard of sanitary practices will result in heavily contaminated milk that spoils rapidly. However, milking performed under hygienic conditions with strict attention to sanitary practices will result in a product with low bacterial content and good keeping quality.

We shall discuss the microorganisms found in milk on the basis of their major characteristics, namely:

1 Biochemical types
2 Temperature response
3 Ability to cause infection and disease

Biochemical Types of Bacteria in Milk

If maintained under conditions that permit bacterial growth, raw milk of a good sanitary quality will develop a clean, sour flavor. This change is brought about mainly by *Streptococcus lactis* and *S. cremoris* (Fig. 28-1A, B) and certain lactobacilli (Fig. 28-1C). The principal change is lactose fermentation to lactic acid; evidence of proteolysis or lipolysis is not detectable by taste or smell. This type of change is sometimes referred to as the normal fermentation of milk. However, other organisms may produce changes beyond mere production of acid as shown in Table 28-1.

Figure 28-1. *Streptococcus lactis* (A) and *S. cremoris* (B), two important fermentative bacterial species in milk and milk products. These species, along with *Lactobacillus fermenti* (C), cause the so-called normal fermentation of milk; they are not pathogens. *(Courtesy of S. Orla-Jensen, The Lactic Acid Bacteria, Ejnar Munksgarrd, Copenhagen, 1919.)* (C) *Lactobacillus fermenti*, one of the heterofermentative lactobacilli. It produces a mixture of acids and is involved in the normal fermentation of milk; it is not pathogenic. Its cells vary in length and are Gram-positive, nonmotile, and nonsporeforming. *(Courtesy of A. P. Harrison.)*

Temperature Characteristics of Bacteria in Milk

Bacteria that gain entrance into milk may be classified according to their optimum temperature for growth and heat resistance. Temperature is a very practical consideration, since low temperatures are used to prevent changes due to microorganisms and high temperatures (pasteurization) are used to reduce the microbial population, destroy pathogens, and in general improve the keeping quality of the milk. Collectively, the bacteria encountered in milk are of the following four temperature types: psychrophilic, mesophilic, thermophilic, and thermoduric.

Since certain psychrophiles grow at temperatures just above freezing and some thermophiles grow at temperatures in excess of 65°C, it follows that the temperature at which milk is held will determine which species grow and predominate. Pasteurized milk stored in a refrigerator may be satisfactorily preserved for a week or even longer. But eventually microbial deterioration, manifested by "off" flavor or odor, will become evident because of the accumulation of metabolic products of psychrophilic bacteria. Thermophiles present a problem at the other extreme of the temperature scale. The holding method of pasteuri-

Table 28-1. Biochemical Types of Microorganisms in Milk

Biochemical Types	Representative Microorganisms	Source of Microorganisms	Substrate Acted Upon and End Products	Additional Remarks
Acid producers	[illegible] streptococci, e.g., *Streptococcus lactis* [illegible]	Dairy utensils, [illegible] plants	[illegible] fermented to lactic acid and other [illegible] acetic acid, [illegible] and carbon [illegible]	Acid producers that produce only lactic acid are referred to as *homofermentative* types; those which produce a variety of products are called *heterofermentative* types
	Lactobacilli, e.g., *Lactobacillus casei* *L. plantarum* *L. brevis* *L. fermentum*	Feeds, silage, [illegible]	[illegible] to lactic [illegible] products. [illegible] lactobacilli [illegible]tive; others [illegible]ntative	
	Microbacteria, e.g., *Microbacterium lacticum*	Manure, dairy utensils, and dairy products	[illegible] fermented to lactic acid and other end products; do not produce as much acid as the streptococci or lactobacilli	Some of these bacteria can survive exposure to very high temperatures, e.g., 80–85°C for 10 min
	Coliforms, e.g., *Escherichia coli* *Enterobacter aerogenes*	Manure, polluted water, soil, and plants	Lactose fermented to a mixture of end products, e.g., acids, gases, and neutral products	The number of coliform bacteria present in milk is an indicator of its sanitary quality
	Micrococci, e.g., *Micrococcus luteus* *M. varians* *M. freudenreichii*	Ducts of cow's mammary glands, dairy utensils	[illegible] products [illegible] produced from lactose (weakly fermentative); micrococci are also weakly proteolytic	Moderately heat resistant; some strains capable of surviving 63°C for 30 min
Gas Producers	Coliforms *Clostridium butyricum* *Torula cremoris*	Soil, manure, water, feed	Lactose fermented with accumulation of gas; the gas may be a mixture of carbon dioxide and hydrogen, or only carbon dioxide in the case of yeast fermentation	Bulk containers of milk may have their lids lifted by gas pressure in instances where contamination with gas producers is unusually high.
Ropy or stringy fermentation	*Alcaligenes viscolactis* *Enterobacter aerogenes* *Streptococcus cremoris*	Soil, water, plants, feed	Organisms synthesize a viscous polysaccharide material that forms a slime layer or capsule on the cells	Milk favors the formation of capsular material; sterile skim milk is frequently used as the culture medium when capsule formation is sought
Proteolytic	*Bacillus* spp., e.g., *B. subtilis* *B. cereus* *Pseudomonas* spp. *Proteus* spp. *Streptococcus liquefaciens*	Soil, water, utensils	Proteolytic organisms degrade the casein to peptides which may be further dissimilated to amino acids; proteolysis may be preceded by coagulation of the casein by the enzyme renin	End products of proteolysis may impart abnormal flavor or odor to the milk; *Pseudomonas* spp. may produce coloration of milk.
Lipolytic	*Pseudomonas fluorescens* *Achromobacter lipolyticum* *Candida lipolytica* *Penicillium* spp.	Soil, water, utensils	Lipolytic microorganisms hydrolyze milk fat to glycerol and fatty acids	Some fatty acids impart rancid odor and taste to milk.

zation exposes milk to a temperature of 62.8°C for 30 min; the thermophile *Bacillus stearothermophilus*, however, grows at 65°C. Other generalizations relating to bacterial growth and the types of bacteria that predominate in milk held at various temperatures are shown in Table 28-2.

In the dairy industry, thermoduric bacteria are regarded as those which survive pasteurization in considerable numbers but do not grow at pasteurization temperatures. Microorganisms of this category are extremely troublesome from the standpoint of producing raw milk with a low bacterial count. Because they are not killed by pasteurization, the microorganisms may contaminate equipment and accumulate as a result of faulty cleaning. Subsequent batches of milk processed through the same equipment will become heavily contaminated.

Thermoduric bacteria are not restricted to a single species or genus but are found in species of several genera, e.g., *Microbacterium lacticum*, *Micrococcus luteus*, *Streptococcus thermophilus*, and *Bacillus subtilis*.

Pathogenic Types of Bacteria in Milk

In recent years milk has been involved in fewer and fewer outbreaks of illness, to the point that the public and regulatory agencies no longer consider milk a primary source of foodborne illness. Milk and dairy products can now be considered model foods from the standpoint of regulations and surveillance of production, processing, and distribution. Furthermore, there are companion standardized methods for analysis of dairy products. No other food can claim the degree of standardized surveillance and analysis that is practiced for milk and milk products.

A variety of diseases are potentially transmissible through milk. The source of a pathogenic agent occurring in milk may be either a cow or a human, and

Table 28-2. Effect of Holding Temperature of Raw Milk on Numbers and Types of Bacteria

Holding Temperature, °C	Changes in Numbers	Predominant Organisms
1–4	Slow decline first few days followed by gradual increase after 7 to 10 days	True psychrophiles, e.g., species of *Flavobacterium*, *Pseudomonas*, and *Alcaligenes*
4–10	Little change in number during first few days followed by rapid increase in numbers; large populations present after 7 to 10 days or more	As above; changes produced on holding are of the following types: ropiness, sweet curdling, proteolysis, etc.
10–20	Very rapid increase in numbers; excessive populations reached within few days or less	Mainly acid-producing types such as lactic streptococci
20–30	High populations develop within hours	Lactic streptococci, coliforms, and other mesophilic types; in addition to acid there may be gas, off flavors, etc.
30–37	High populations develop within hours	Coliform group favored
Above 37	High populations develop within hours	Some mesophiles, thermophiles, e.g., *Bacillus coagulans* and *B. stearothermophilus*

it may be transmitted to either. The following modes of transmission are possibilities.

1 Pathogen from infected cow → milk → human or cow, e.g., tuberculosis, brucellosis, mastitis
2 Pathogen from human (infected or carrier) → milk → human, e.g., typhoid fever, diphtheria, dysentery, scarlet fever

It is also possible for humans to infect cows. For example, mastitis may be caused by a variety of organisms, including *Staphylococcus aureus*. The infecting organism, in some cases, has been traced to humans.

More specific aspects of disease transmission by milk and other foods are discussed in Part Eight.

MICROBIAL SPOILAGE OF FOODS

Considering the variety of natural food substances and the methods by which each is handled during processing, it is apparent that practically all kinds of microorganisms are potential contaminants. The type of food substance and the method by which it is processed and preserved may favor contamination by certain groups of microorganisms. Most foodstuffs serve as good media for the growth of many different microorganisms. Given a chance to grow, the organisms will produce changes in appearance, flavor, odor, and other qualities of foods. These degradation processes may be described as follows:

Putrefaction:
Protein foods + proteolytic microorganisms → amino acids + amines + ammonia + hydrogen sulfide

Fermentation:
Carbohydrate foods + carbohydrate-fermenting microorganisms → acids + alcohols + gases

Rancidity:
Fatty foods + lipolytic microorganisms → fatty acids + glycerol

The changes that microbes cause in foods are not limited to the results of degradation; they may also be caused by products of microbial synthesis. Some microorganisms discolor foods as a result of pigment production. Slimes may be developed in or on foods by microorganisms capable of synthesizing certain polysaccharides.

Fresh Foods

Examples of types of food spoilage (other than canned-food spoilage), together with some of the microorganisms involved, are shown in Table 25-3.

Fresh Milk

Milk is an excellent bacteriological medium. In fact, sterile skimmed milk is used routinely as a culture medium. Fresh whole milk contains protein (casein), carbohydrate (lactose), and fat. All of these substrates can be degraded enzymatically by microorganisms. If the degradation of these substrates is extensive, the accumulation of end products will impart undesirable characteristics to the milk. Some microorganisms can synthesize compounds like pigments and slimes which also give undesirable characteristics to the milk. A summary of

Table 28-3. Types of Food Spoilage (Other than Canned Foods) with Some Examples of Causative Organisms

Food	Type of Spoilage	Some Microorganisms Involved
Bread	Moldy	*Rhizopus nigricans* *Penicillium* *Aspergillus niger*
	Ropy	*Bacillus subtilis*
Maple sap and syrup	Ropy	*Enterobacter aerogenes*
	Yeasty	*Saccharomyces* *Zygosaccharomyces*
	Pink	*Micrococcus roseus*
	Moldy	*Aspergillus* *Penicillium*
Fresh fruits and vegetables	Soft rot	*Rhizopus* *Erwinia*
	Gray mold rot	*Botrytis*
	Black mold rot	*A. niger*
Pickles, sauerkraut	Film yeasts, pink yeasts	*Rhodotorula*
Fresh meat	Putrefaction	*Alcaligenes* *Clostridium* *Proteus vulgaris* *Pseudomonas fluorescens*
Cured meat	Moldy	*Aspergillus* *Rhizopus* *Penicillium*
	Souring	*Pseudomonas* *Micrococcus*
	Greening, slime	*Lactobacillus* *Leuconostoc*
Fish	Discoloration	*Pseudomonas*
	Putrefaction	*Alcaligenes* *Flavobacterium*
Eggs	Green rot	*P. fluorescens*
	Colorless rots	*Pseudomonas* *Alcaligenes*
	Black rots	*Proteus*
Concentrated orange juice	"Off" flavor	*Lactobacillus* *Leuconostoc* *Acetobacter*
Poultry	Slime, odor	*Pseudomonas* *Alcaligenes*

microbial biochemical types that may occur in milk, their source, and the changes they produce are shown in Table 28-1.

Table 28-4. Microbiology of Canned-Food Spoilage

Type of Product	Type of Spoilage Organisms, with Examples	Signs of Spoilage: Can	Signs of Spoilage: Contents of Can
Low and medium acid products, pH above 4.6, e.g., corn, peas, spinach, asparagus	Flat sour (*Bacillus stearothermophilus*)	Possible loss of vacuum on storage	Appearance not usually altered; pH markedly lowered; sour; may have slightly abnormal odor; sometimes cloudy liquor
	Thermophilic anaerobe (*Clostridium thermosaccharolyticum*)	Can swells, may burst	Fermented, sour, cheesy, or butyric odor
	Sulfide spoilage (*Clostridium nigrificans*)	Can flat, hydrogen sulfide gas absorbed by product	Usually blackened, "rotten egg" odor
	Putrefactive anaerobe (*Clostridium sporogenes*)	Can swells, may burst	May be partially digested; pH slightly above normal; typical putrid odor; may be toxic
	Aerobic sporeformers (odd types) (*Bacillus* spp.)	Usually no swelling, except in cured meats when nitrate and sugar are present	Coagulated evaporated milk, black beets
Acid products, pH below 4.6, e.g., tomato juice, fruits, and fruit juices	Flat sour (*Bacillus thermoacidurans*)	Can flat, little change in vacuum	Slight pH change; off odor and flavor
	Butyric anaerobes (*Clostridium butyricum*)	Can swells, may burst	Fermented, butyric odor
	Nonsporeformers (mostly lactic acid types of bacteria)	Can swells, usually bursts, but swelling may be arrested	Acid odor
	Yeasts	Can swells, may burst	Fermented; yeasty odor
	Molds	Can flat	Surface growth; musty odor

SOURCE: Data from the National Food Processors Association.

Canned Foods

Because of their heat resistance, sporeformers (species of *Clostridium* and *Bacillus*) constitute the most important group of microorganisms in the canning industry. The three most important types of microbiological spoilage of commercially canned foods are (1) flat sour spoilage, (2) thermophilic anaerobe (TA) spoilage, and (3) putrefaction. Table 28-4 presents a summary of organisms involved in spoilage of canned food, together with the changes they produce.

MICROBIOLOGICAL EXAMINATION OF FOODS

Microbiological examination of foods may provide information concerning the quality of the raw food and the sanitary conditions under which the food was processed as well as the effectiveness of the method of preservation. In the case of spoiled foods, it is possible to identify the agent responsible for the spoilage; having discovered the agent, it may be possible to trace the source of contamination and the conditions which permitted spoilage to occur. Corrective measures can then be instituted to prevent further spoilage.

Microbiological food examination takes advantage of special microscopic techniques and cultural procedures. Extensive use is made of selective and differential media to facilitate the enumeration and isolation of certain types of microorganisms. The particular procedure used is determined by the type of food product being examined as well as by the specific purpose of the examination. For example, a food sample being investigated for possible contamination by *Clostridium botulinum* would be subject to different laboratory tests than one being examined for coliform organisms. The increasing significance of salmonellas in foodborne disease has made it mandatory to develop more rapid, reliable, and reproducible methods for the detection of salmonellas in foods.

A schematic summary of the various approaches to consider in the microbiological examination of a food sample is shown in Fig. 28-2. The procedures selected for examination of a particular sample are, of course, determined by the facts relating to that sample and the purpose of the examination.

Microscopic Techniques

Standard microscopic techniques are available for the examination of some food products. For example, a procedure known as the Breed smear is used to make a direct microscopic count of microorganisms in milk. The essential procedures of this technique are: (1) spreading a measured amount of milk over a known area on a glass slide, (2) staining the film of milk with methylene blue, (3) making a microscopic count of organisms or clumps of organisms in several microscopic fields, and (4) calculating the total number of bacteria per unit volume.

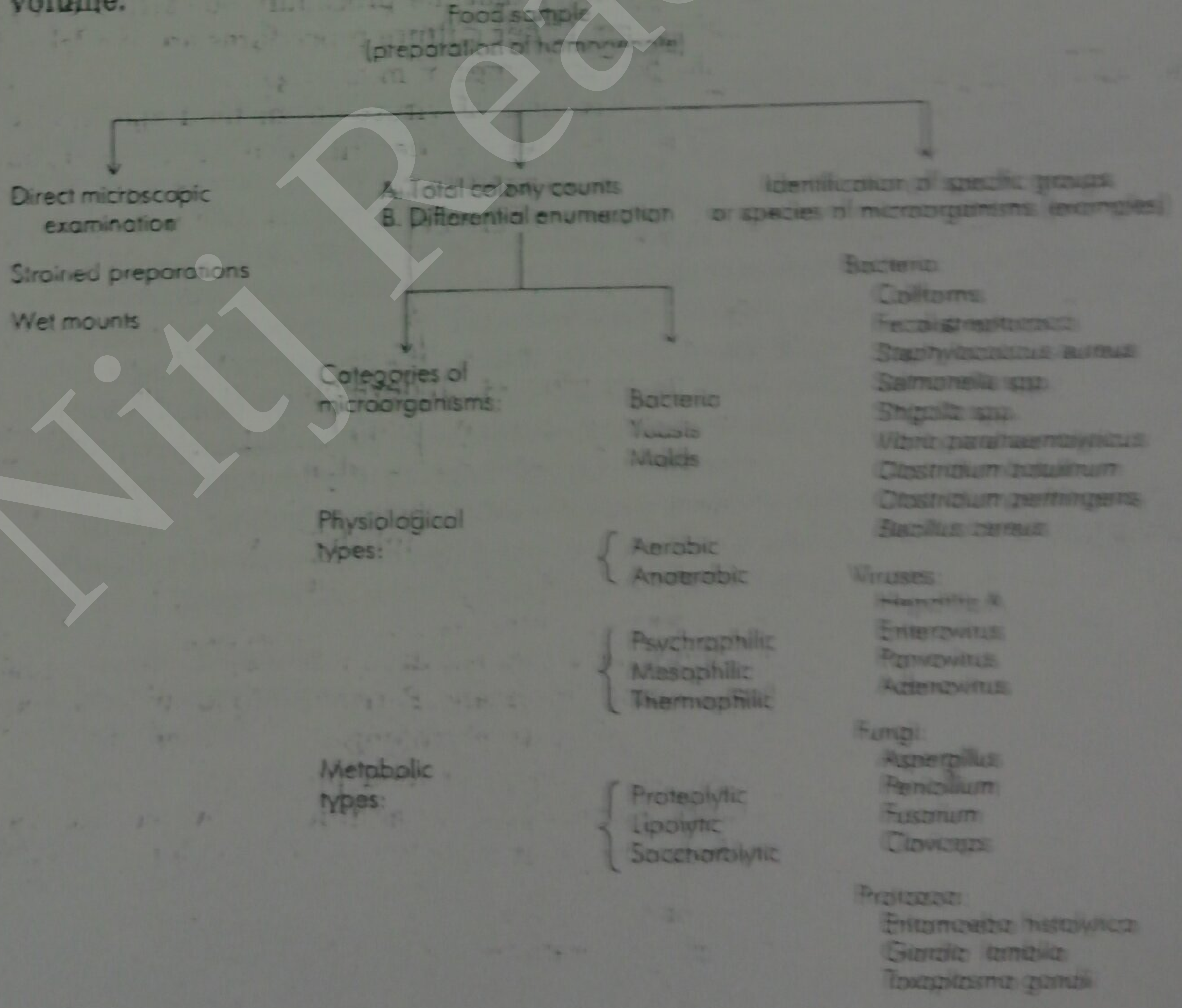

Figure 28-2. Generalized scheme for microbiological examination of foods.

A slide designed with a chamber, known as the Howard mold slide, is used, as its name suggests, to enumerate mold filaments in food products such as fruits, juices, and vegetables. When the mold counts obtained by this procedure exceed certain limits, it indicates raw material of poor quality or unsatisfactory sanitary processing.

Protozoa can be identified and enumerated by direct microscopic examination. Since the protozoa may be present in small numbers, it is frequently necessary to use a procedure which will concentrate these organisms in the food sample prior to microscopic examination.

Culture Techniques

The numerous techniques for cultivating microorganisms described in earlier chapters of this book find application, sometimes with modifications, for the examination of foods. For example, plate culture techniques are available for the enumeration of the "total" microbial population or some particular group of microorganisms, as illustrated in Fig. 28-2. The word *total*, of course, needs qualification; the microorganisms enumerated by a cultural technique are only that portion of the total population which will grow into colonies under the conditions provided, namely, the composition of the medium and the physical conditions of incubation. For example, the standard procedure for counting microorganisms in milk is designed to enumerate bacteria by the standard plate count (SPC). The conditions for the procedure are very specifically articulated in a volume entitled *Standard Methods for the Examination of Dairy Products*. It is mandatory that the procedure be carried out precisely as specified in the publication. Other culture procedures are available for particular physiological or biochemical types of microorganisms.

The cultivation of viruses from food specimens requires the use of tissue-culture techniques as described in Chap. 21. Prior concentration of the food specimen suspected to be contaminated with viruses may be necessary. Additional provisions are necessary to inhibit bacterial growth in the tissue culture.

PRESERVATION OF FOODS

Today we associate food preservation with the refrigerator, the deep freeze, and the canning process, all developments of the nineteenth and twentieth centuries. However, humans have grappled with the problem of food preservation for many centuries. The ancient Egyptians and Romans were aware of the preservative effects of salting, drying, and smoking. It has been suggested that the first salt preservation was accomplished by burying the food along the shore, where seawater effected the cure. The American Indians placed strips of fresh bison and venison at the top of a teepee or over a campfire, where preservation was accomplished through drying and smoking. Dried salt cod was a common food for colonial Americans. Perishable foods were stored in caves and springs, where the low temperature prolonged the preservation.

Modern methods of food preservation employ elaborate refinements of the primitive processes plus additional new techniques. The various practices used for food preservation may be summarized as follows:

1 Aseptic handling
2 High temperatures
    (a) Boiling

- (b) Steam under pressure
  - (c) Pasteurization
  - (d) Sterilization (of milk)
  - (e) Aseptic processing
- 3 Low temperatures
  - (a) Refrigeration
  - (b) Freezing
- 4 Dehydration
- 5 Osmotic pressure
  - (a) In concentrated sugar
  - (b) With brine
- 6 Chemicals
  - (a) Organic acids
  - (b) Substances developing during processing (smoking)
  - (c) Substances contributed by microbial fermentation (acids)
- 7 Radiation
  - (a) Ultraviolet
  - (b) Ionizing radiations

All methods of food preservation are based upon one or more of the following principles: (1) prevention or removal of contamination, (2) inhibition of microbial growth and metabolism (microbistatic action), and (3) killing of microorganisms (microbicidal action).

septic Handling

Food items undergo considerable handling prior to being processed by some specific method of preservation such as canning, freezing, or dehydration. Each step in the preparation of a food for its final treatment is a potential source of contamination. For example, the shell of an egg provides a protective covering which normally excludes microorganisms. However, when the eggs are cracked open in the process of preparing dehydrated egg powder it is likely that the interior of the egg may become contaminated. The extent of the contamination will depend upon the cleanliness of the eggs and the level of aseptic precautions observed in the process.

One can recognize more vividly the importance of asepic technique in the processing of more perishable foods like oysters and crabmeat, each of which requires considerable handling by people.

gh Temperatures

High temperature is one of the safest and most reliable methods of food preservation. Heat is widely used to destroy organisms in food products in cans, jars, or other types of containers that restrict the entrance of microorganisms after processing.

Steam under pressure, such as in a pressure cooker, is the most effective method of high-temperature food preservation since it can kill all vegetative cells and spores. Food preservation by heat requires knowledge of the heat resistance of microorganisms, particularly spores. In addition, one must consider the rate at which heat penetrates through foods of different consistencies as well as the size of the containers in which they are packed. Killing microorganisms by heat involves a time-temperature relationship, as discussed in Chap. 22, and considerable experimentation has been performed to determine the thermal death times of bacteria likely to cause spoilage. From such information it is possible to establish satisfactory heat-processing conditions. Much research has been done on this subject, and this accounts for the highly successful results achieved in food preservation by canning. Special laboratory equipment has been designed to determine with precision the heat resistance of various bacterial species, particularly the sporeformers.

Canning

Canning has been the basic method of food preservation for approximately 175 years. In 1810 Nicholas Appert, a Frenchman, published *L'Art de Conserver*, which described his successful researches in food preservation; and in the same year Peter Durand was granted an English patent describing the use of tin containers for food preservation.

The temperatures used for canning foods ranges from 100°C for high-acid foods to 121°C for low-acid foods. The canning process does not guarantee a sterile product. For example, spores of some bacterial species may survive these temperatures.

The most important organism to be eliminated in canned foods is the spore-forming anaerobe *Cl. botulinum*, which is capable of producing a very potent lethal toxin.

Pasteurization of Milk

The "Milk Ordinance and Code" of the U.S. Public Health Service comments on the word *pasteurization* as follows:

> The terms *pasteurization*, *pasteurized*, and similar terms shall mean the process of heating every particle of milk or milk product to at least 145°F., and holding it continuously at or above this temperature for at least 30 minutes, or to at least 161°F., and holding it continuously at or above this temperature for at least 15 seconds, in equipment which is properly operated and approved by the health authority . . . .

The original time-temperature relationships for pasteurization were worked out with *Mycobacterium tuberculosis* since this was regarded as the most heat-resistant pathogen likely to occur in milk (see Fig. 28-3). This organism is

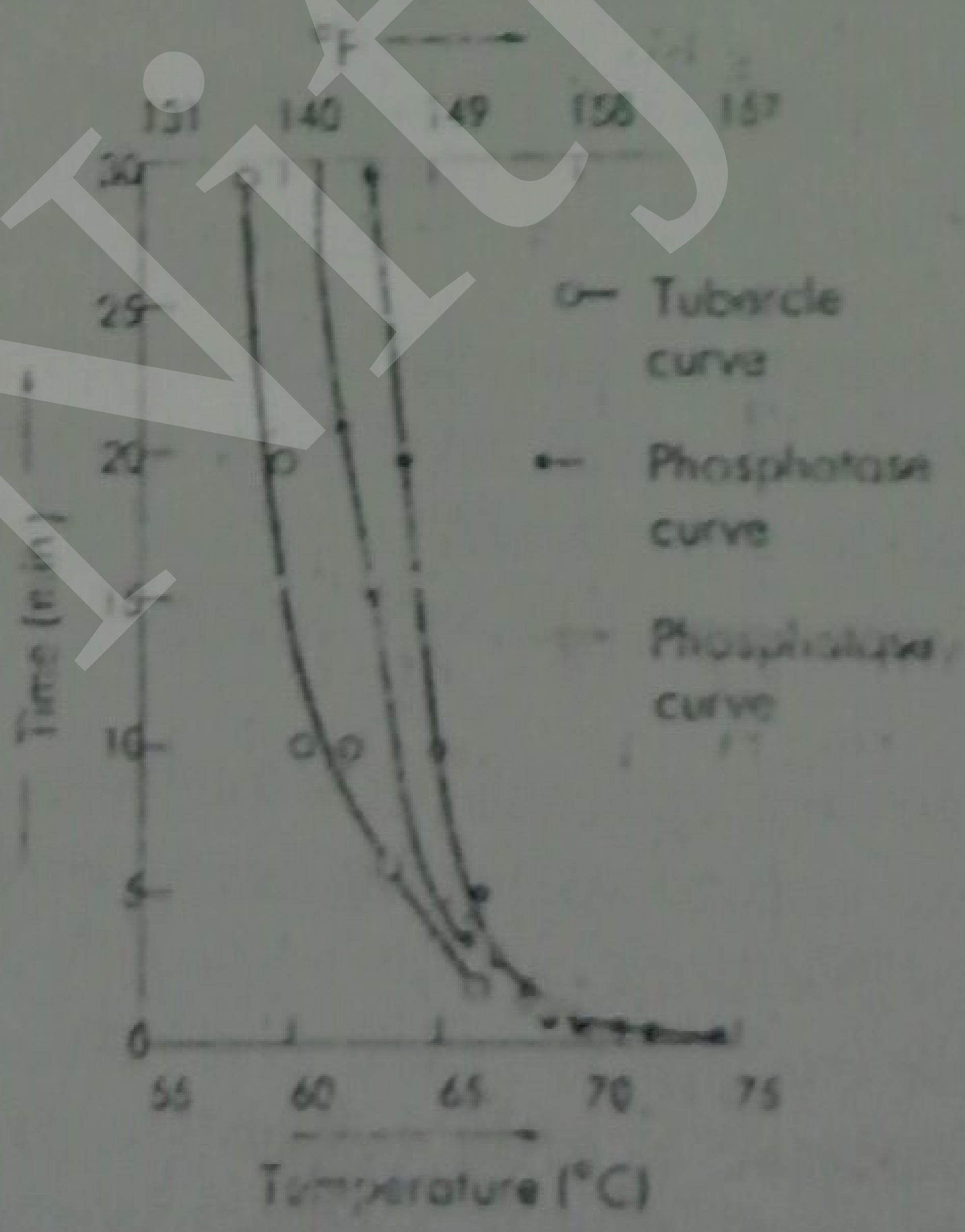

Figure 28-3. Time-temperature curve for the killing of *Mycobacterium tuberculosis* compared with the time and temperature required for the inactivation of the enzyme phosphatase. The two phosphatase curves are plotted from different experimental data. (Courtesy of *McGraw-Hill Encyclopedia of Science and Technology*, p. 562, vol. [illegible] Copyright 1971. McGraw-Hill Book Company.)

destroyed when exposed to a temperature of 140°F for 10 min. The pasteurization temperature was set at 143°F for 30 min. Later it was discovered that *Coxiella burnetii*, the causative agent of Q fever which can be transmitted by milk, can survive in milk heated to 143°F for 30 min. This observation resulted in the establishment of the present time and temperature for pasteurization.

Pasteurization Processes. Methods of pasteurization of milk used commercially include a low-temperature holding (LTH) method and a high-temperature short-time (HTST) method. The holding method, or vat pasteurization, exposes milk to 145°F (62.8°C) for 30 min in appropriately designed equipment. The HTST process employs equipment capable of exposing milk to a temperature of 161°F (71.7°C) for 15 s (seconds). In either method of pasteurization it is essential that the equipment be designed and operated so that every particle of milk is heated to the required temperature and held for the specified time. Precautions must be taken to prevent recontamination after pasteurization. The finished product should be stored at a low temperature to retard growth of microorganisms which survived pasteurization.

In addition to milk numerous other food products and some fermented beverages like beers and wines are commercially pasteurized.

The Phosphatase Test. Phosphatase is an enzyme, present in raw milk and in many tissues, which is destroyed by adequate pasteurization (see Fig. 28-3). Thus one can determine whether milk has been properly pasteurized by testing for the absence of this enzyme. The principle of the test is illustrated by the following reaction. Milk, which in its raw condition contains the enzyme phosphatase, is added to a substrate upon which the enzyme will react:

Disodiumphenyl phosphate + phosphatase → phenol + sodium phosphate
Substrate — Enzyme from raw milk — Products

The amount of phenol liberated can be conveniently estimated by the addition of a reagent which turns blue in the presence of phenol. Color standards are used to interpret the results of this test. This is a very simple testing procedure, yet it provides valuable information about the heat treatment milk has received.

Sterilization

Commercial milk-sterilization techniques have been developed which expose milk to ultrahigh temperatures for very short periods of time, for example, 300°F (148.9°C) for 1 to 2 s. In addition, the sterilization process includes steps that eliminate any traces of cooked flavor. The final product is comparable in flavor and nutritional quality to pasteurized milk. The sterile milk product has several attractive features: it does not require refrigeration and it has an indefinite shelf life.

Aseptic Processing

A relatively new commercial development in the food industry is known as aseptic processing. The food item is commercially sterilized and packaged into previously sterilized containers under aseptic conditions.

This process has the advantage that it uses containers other than cans. This provides significant economic and user advantages.

Temperatures approaching 0°C and lower retard the growth and metabolic activities of microorganisms. Modern refrigeration and freezing equipment has made it possible to transport and store perishable foods for long periods of time. Refrigerated trucks and railway cars, ships' storage vaults, and the home refrigerator and freezer have improved the quality of the human diet and increased the variety of foods available. Frozen-food production in the United States almost doubled from 11 billion pounds in 1965 to 20 billion in 1975 and is expected to more than double to 48 billion pounds by 1985. Much of this increase will be in prepared frozen foods, whose quantity tripled over the last 10 years and is expected to approach 50 percent of all frozen foods by 1985. The growth and importance of this segment of the food industry places greater emphasis on the study of microorganisms at low temperatures, e.g., their survival, growth, and metabolic activity.

Before freezing, the fresh produce is steamed (blanched) to inactivate enzymes that would alter the product even at low temperatures. Quick-freeze methods, using temperatures of −32°C or lower, are considered most satisfactory; smaller crystals of ice are formed, and cell structures in the food are not disrupted. It should be emphasized that freezing foods, no matter how low the temperature, cannot be relied upon to kill all microorganisms. The number and types of viable and nonviable microorganisms present in frozen foods reflect the degree of contamination of the raw product, the sanitation in the processing plant, and the speed and care with which the product was processed. The microbial count of most frozen foods decreases during storage; but many organisms, including pathogens, e.g., species of *Salmonella*, survive for long periods of time at −9 and −17°C. The temperature ranges at which food-poisoning bacteria and psychrophilic microorganisms are capable of growing are shown in Fig. 28-4.

active →

inactive

The increased use of precooked ready-to-serve foods and the prevalence of automatic vending machines for dispensing perishable foods have made it necessary to obtain more data on microbial growth and survival at low temperatures. Figure 28-5 illustrates the growth of salmonellas and staphylococci in prepared foods at various temperatures and times of incubation. Note that the type of

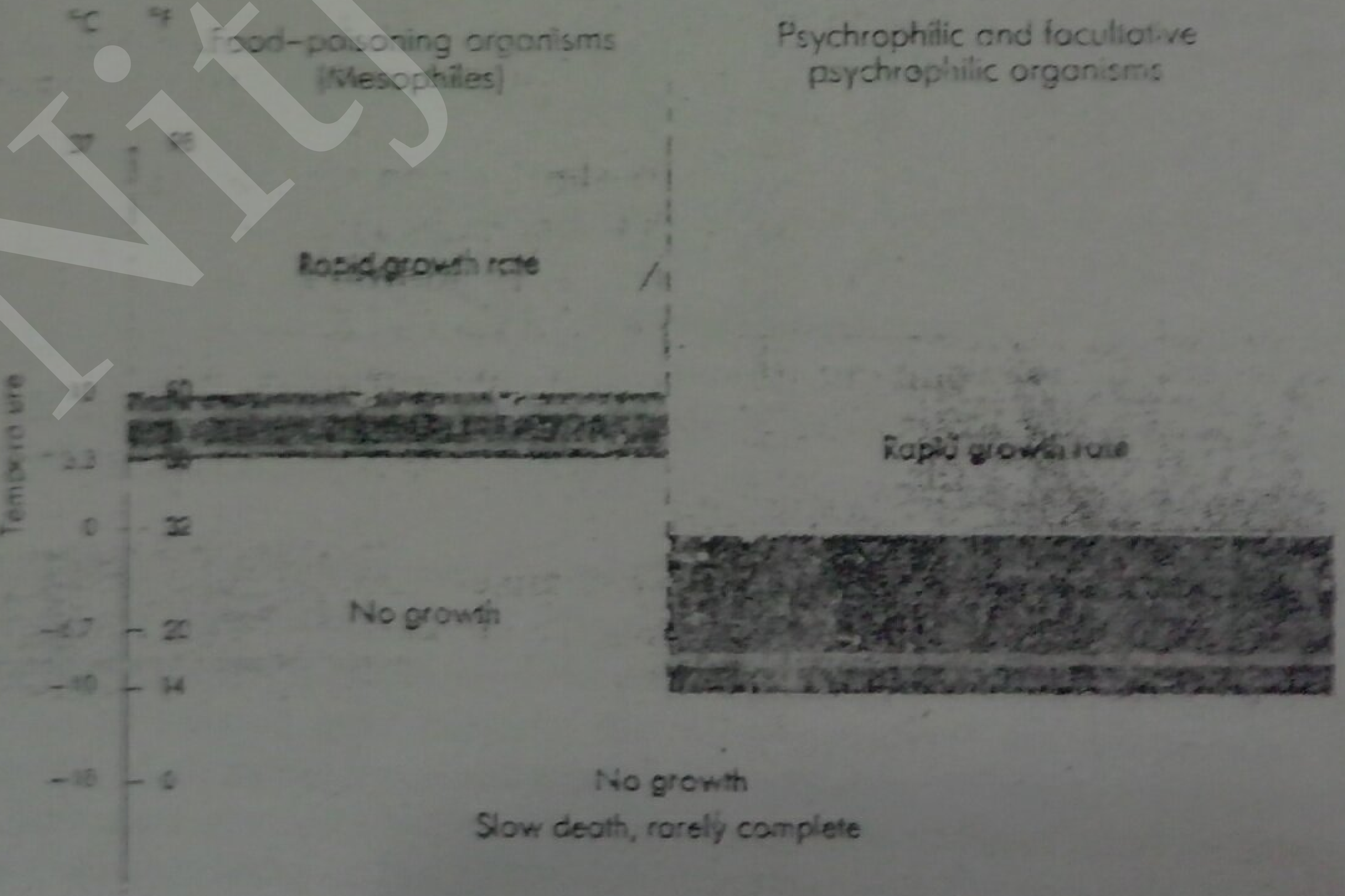

Figure 28-4. Food-poisoning organisms grow in a somewhat higher temperature range than psychrophilic microorganisms. (Courtesy of R. P Elliot and H. D. Michener, *Review of the Microbiology of Frozen Foods,* in "Conference on Frozen Food Quality," ARS-74-21, USDA, 1960.)

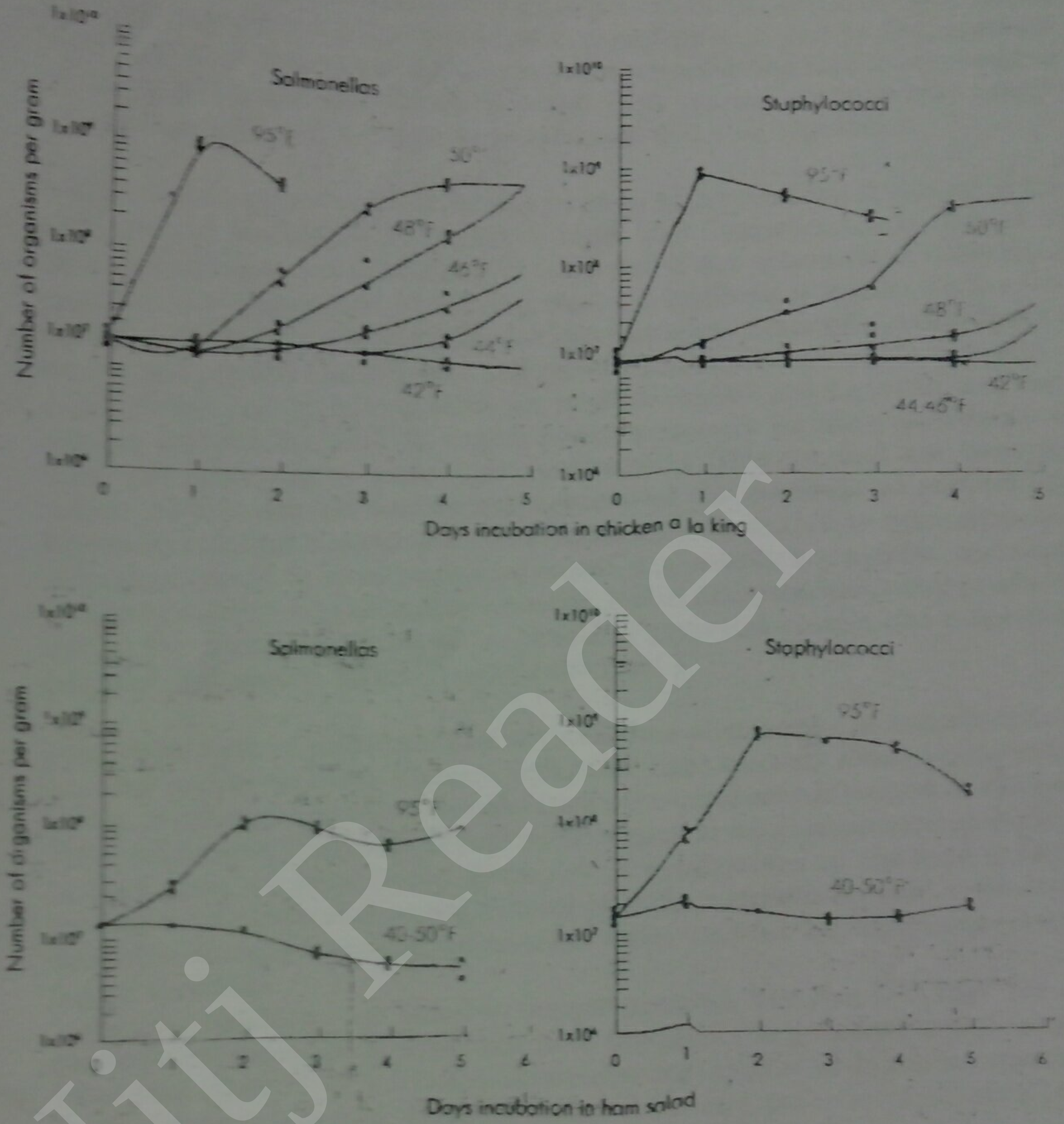

Figure 28-5. Salmonellas and staphylococci multiply rapidly in chicken a la king and ham salad incubated at room temperature. Curves also show growth at other temperatures. (Courtesy of *R. Angelotti, M. J. Foter, and K. H. Lewis, "Time-Temperature Effects on Salmonellae and Staphylococci in Foods,"* Am J Public Health, 51:76–88 1961.)

food product has considerable influence on the rate of bacterial growth at the lower temperatures.

Dehydration

Dried foods have been used for centuries, and they are more common throughout the world than frozen foods. The removal of water by drying in the sun and air or with applied heat causes dehydration. The preservative effect of dehydration is due mainly to microbistasis; the microorganisms are not necessarily killed. Growth of all microorganisms can be prevented by reducing the moisture content of their environment below a critical level. The critical level is determined by the characteristics of the particular organism and the capacity of the food item to bind water so that it is not available as free moisture. It will be recalled that lyophilized cultures of microorganisms survive for years.

Osmotic Pressure

Water is withdrawn from microorganisms placed in solutions containing large amounts of dissolved substances such as sugar or salt. The cells are plasmolyzed, and metabolism is arrested. Thus the antimicrobial condition imposed by increased osmotic pressure is similar in principle to inhibition by dehydration. Although yeasts and molds are relatively resistant to osmotic changes, processes of food preservation based on this principle are, nevertheless, very useful. Jellies and jams are rarely affected by bacterial action because of high sugar content. However, it is not uncommon to find mold growth on the surface of jelly which has been exposed to air. Condensed milk is preserved in part by the increased concentration of lactose and supplemental sucrose. Similar results are obtained by curing meats and other foods in brines. High osmotic pressure may inhibit microbial growth, but it cannot be relied upon to kill all organisms.

Chemicals

Addition of chemicals to foods for the purpose of preservation is subject to the provisions of the United States Food, Drug, and Cosmetic Act as revised in 1972. According to this act, a food is adulterated if any poisonous or deleterious substance has been added which may render it injurious to health. Only a few chemicals are legally acceptable for food preservation. Among the most effective are benzoic, sorbic, acetic, lactic, and propionic acids, all of which are organic acids. Sorbic and propionic acids are used to inhibit mold growth in bread. Nitrates and nitrites used in curing meats, primarily for the preservation of color, are inhibitory to some anaerobic bacteria. This practice has been the subject of considerable controversy because of the potential of nitrates and nitrites as mutagenic agents and the subsequent relationship to carcinogenesis. [Foods prepared by fermentation processes, e.g., sauerkraut, pickles, and silage for animals, are preserved mainly by acetic, lactic, and propionic acids produced during the microbial fermentation. Smoking generates cresols and other antibacterial compounds which penetrate the meat.]

Radiation

Ultraviolet light of sufficient intensity and time of exposure is microbicidal to exposed microorganisms. Because ultraviolet light has very limited penetration power, microorganisms that are embedded or covered are unlikely to be affected. Thus, ultraviolet irradiation is limited to control of microorganisms on surfaces or thin, clear layers of liquid. Examples of applications in the food industry include meat-processing plants, control of surface growth on bakery products, sanitation of equipment, and treatment of water used for the depuration (cleansing) of shellfish.

Ionizing radiations are lethal to microorganisms. The fact that they are microbicidal at room temperature and have the ability to penetrate are characteristics that make them attractive candidates for control of microorganisms in foods. Gamma rays and electron beams (beta and cathode rays) have been experimented with extensively for use in the food industry.

Canned and packaged foods can be sterilized by an appropriate radiation dosage. This "cold sterilization" produces a rise in temperature of the product of only a few degrees. Radiation pasteurization is a term describing the killing of over 98—but not 100—percent of the organisms by intermediate doses of ionizing radiation.

The ionizing radiation resistance of microorganisms does not correspond to their thermal resistance. *Clostridium botulinum* appears to be the most radioresistant organism of importance to the food technologist. Figure 28-6 illusof the following groups of microorganisms from a sample of food: thermophilic sporeformers, coliforms, and viruses.

17 Compare the microscopic and cultural techniques for microbiological analysis of foods. What are the advantages and limitations of each of these procedures?

18 Name several foods that are prepared by microbial fermentations. Describe the role of microorganisms in each example.

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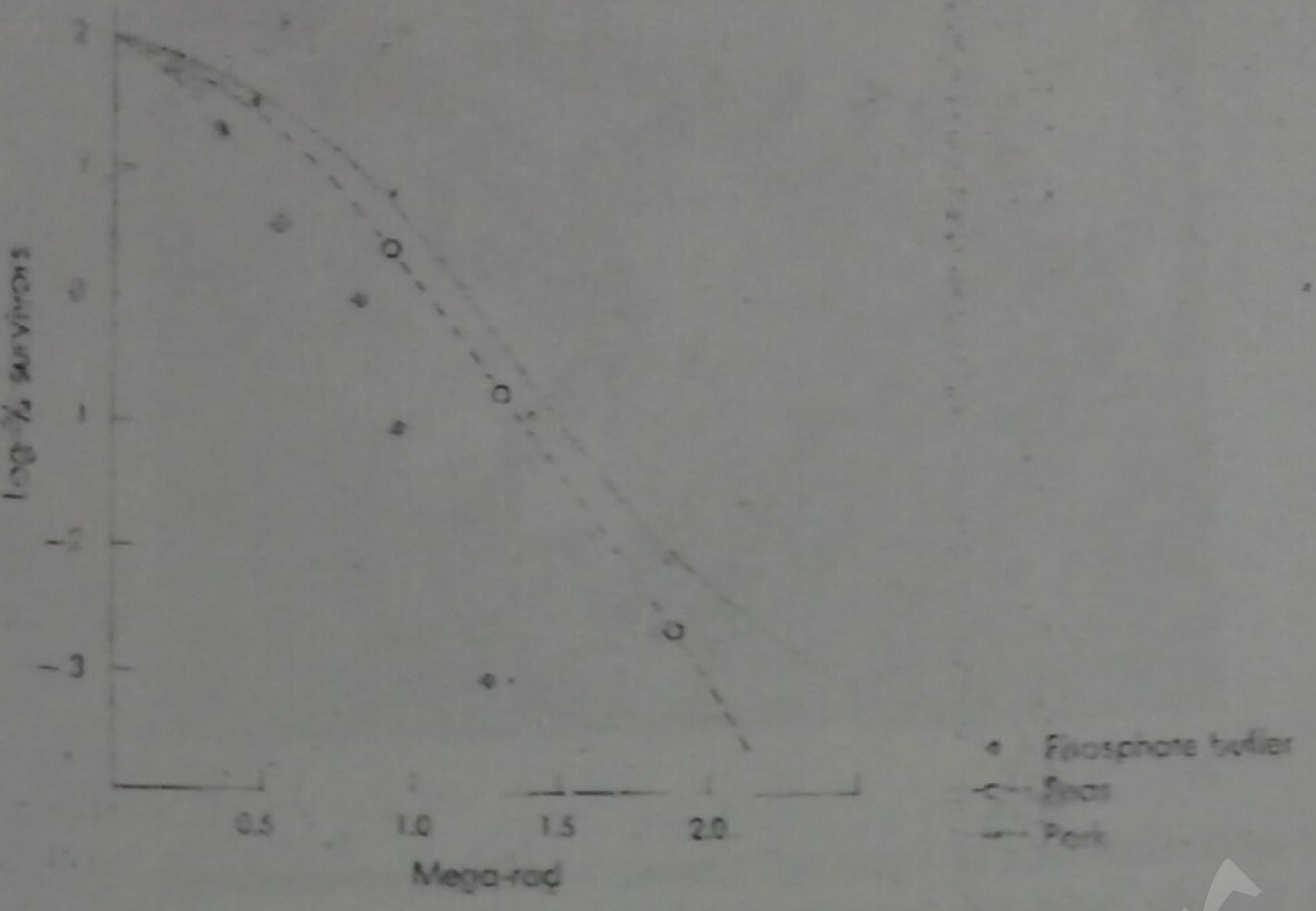

Figure 28-6. Gamma radiation kills spores of *Clostridium botulinum* in frozen foods. Curves show the effect on spores in pork, peas, and phosphate buffer. (Courtesy of C. B. Denny, C. W. Bohrer, W. E. Perkins, and C. T. Townsend, "Destruction of *Clostridium botulinum* by Ionizing Radiation," *Food Res*, 24:44–50, 1959.)

trates the lethal effect of gamma radiation on spores of *C. botulinum*. Note that the survival of spores is influenced by the material in which they are suspended and that time is not a factor. In the case of radiation, unlike temperature, the radiation death dose rather than radiation death time is determined.

Ionizing radiation sterilization provides the possibility of an entirely new approach to food preservation; it could bring about a radical change in industrial methods of food processing. However, despite the extensive research and documentation on the effectiveness of ionization radiation for the preservation of foods, this method of preservation has not been approved in the United States. This is due in part to economic factors as well as to some lingering uncertainties about the effect of the radiation on the food material. In addition, the United States already has well-developed systems for food preservation. This is not the case for all countries. The World Health Organization approved (1976) radiation of poultry at a specified level, as has Canada for controlling salmonellas. In July 1983, the U.S. Food and Drug Administration approved the use of ionizing radiation for sterilization of specific spices and vegetable seasonings.

FERMENTED FOODS

Thus far we have stressed the undesirable characteristics of microorganisms in food. However, there are many useful applications of microorganisms in the food industry. A variety of important products in our diet are produced with the aid of microbial activity.

Fermented Dairy Products

In the dairy industry, fermented milks are produced by inoculating pasteurized milk with a known culture of microorganisms, sometimes referred to as a starter culture, which can be relied on to produce the desired fermentation, thus assuring a uniformly good product. (See Fig. 28-7, which shows *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, organisms used as starter cultures in the preparation of yogurt.)

Several hundred varieties of cheese are manufactured, and with few exceptions, most of them can be made from the same batch of milk. Microorganisms—

Figure 28-7. Photomicrograph of yogurt, illustrating microbial flora, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (X600). (*Courtesy of K. J. Demeter, Bakteriologische Untersuchungsmethoden der Milchwirtschaft, Eugen Ulmer, Stuttgart, 1967.*)

bacteria or molds—convert the curd of the milk into the desired cheese. For the manufacture of some cheeses, such as blue cheese or Roquefort (blue cheese made in Roquefort, France), it is necessary to inoculate the curd with the microorganism which brings about the changes (in this case, *Penicillium roqueforti*). Some of the steps in the process of making Roquefort cheese are shown in Fig. 28-8.

Other Fermented Foods

Important food items produced in whole or in part by microbial fermentations include pickles, sauerkraut, olives, and certain types of sausage. Lactic acid bacteria are chiefly responsible for the desirable type of fermentation required for the production of each of these products. The microorganisms that produce the changes may be the natural flora on the material to be fermented or may be something added as a starter culture. Most commercial sour, sweet, mustard, and mixed pickles are made from fermented salt-stock pickles. The other major type of pickled cucumber is the fermented dill pickle. An illustration of a commercial fermentation process for the production of dill pickles is shown in Fig. 28-9.

The list of food products produced by microbial fermentation is very long. A few examples are shown in Tables 28-5 and 28-6.

MICROORGANISMS AS FOOD—SINGLE-CELL PROTEIN

Bacteria, yeasts, and algae, produced in massive quantities, are attractive sources of food for animals as well as humans. These microorganisms can be cultivated on industrial wastes or by-products as nutrients and yield a large cell crop that is rich in protein (single-cell protein). Bacterial cells grown on hydrocarbon wastes from the petroleum industry are a source of protein in France, Japan, Taiwan, and India. Yeast-cell crops harvested from the vats used to produce

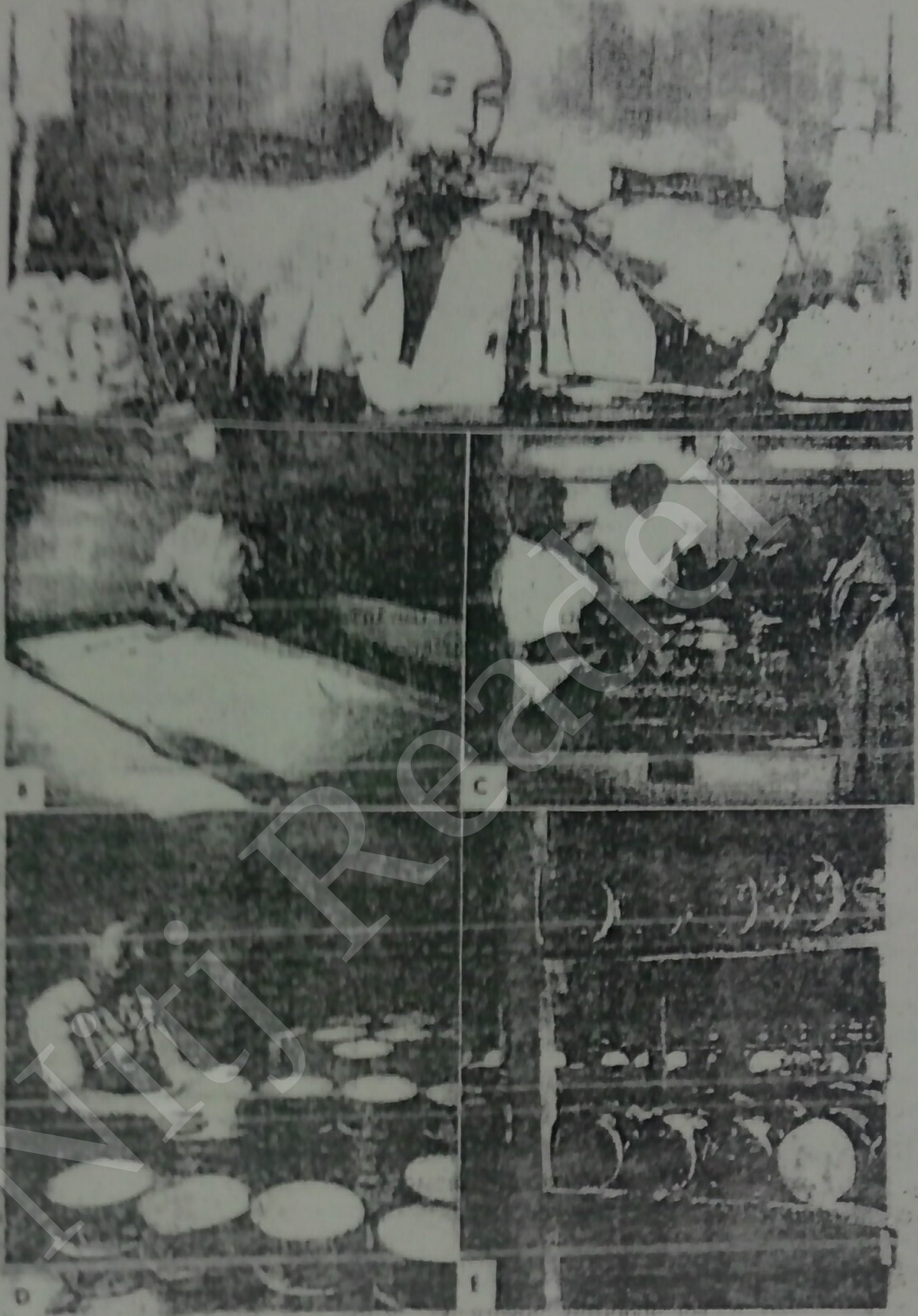

Figure 28-8. Roquefort and blue cheese. (A) Cubes of sterile whole wheat bread are inoculated with *Penicillium roqueforti.* After extensive growth of the mold on the bread cubes, the cubes are removed, dried, and powdered and used as inoculum for making cheese. (Courtesy of the Borden Company.) (B) The addition of a lactic culture and rennet curdles the milk. The curd is cut when it becomes firm. (C) The curd particles are removed and placed in metal hoops. The addition of the spores of *P. roqueforti* may take place in either of these steps. (D) The hoops are placed on a draining board to facilitate whey drainage and matting of the curd, after which the curd is removed, salted periodically, and (E) eventually placed in an area of high humidity (95 to 98 percent) and low temperature (9 to 12°C), where the ripening process occurs over a period of several months. The hoops of cheese shown ripening here are wrapped in foil. [(B to E) Courtesy of Roquefort Association, Inc.]

alcoholic beverages have been used as a food supplement for generations. The attractiveness of single-cell protein as a food substitute or supplement is apparent from the following characteristics of the process.

1 Microorganisms grow very rapidly and produce a high yield. It has been calculated that one can obtain a gain of 1 lb of protein in 1 day's growth from a 1000-lb steer; 1000 lb of yeast would produce several tons of protein in one day! Algae grown in ponds can produce 20 tons (dry weight) of protein per acre per year.

Figure 28-9. (A) Typical [illegible] fermentation and storage of brined cucumbers. The wooden tanks shown are 600- to 1000-bushel capacity. Some pickle companies now use fiberglass tanks. Note the tall, white tank in the background, which is for storage of liquid nitrogen. Nitrogen gas is piped to each brine tank for use in purging of dissolved CO_2 from fermenting brines to prevent bloater damage in the cucumbers. (*Courtesy of H. P. Fleming, USDA.*) (B) Surface of a cucumber brine tank being nitrogen purged with a sidearm purger. Nitrogen gas purges dissolved CO_2 from the brine and also serves to circulate the brine. The white frothing on the surface is caused by the purging action. (*Courtesy of H. P. Fleming, USDA.*)

Table 28-5. Some Characteristics of Fermented Milks

Fermented Product	Principal Microorganisms Responsible for Fermentation	General Remarks
Cultured buttermilk	A mixture of lactic streptococci (*Streptococcus lactis* or *S. cremoris*) with aroma-producing bacteria (*Leuconostoc citrovorum* or *L. dextranicum*)	The function of the lactic acid streptococci is to produce lactic acid that gives the sour taste and to curdle the milk; the function of the leuconostocs is to produce volatile and neutral products that impart a characteristic desirable odor; the starter culture must contain vigorously growing bacteria; incubation is performed at 21°C
Cultured sour cream	Same as used for cultured buttermilk, i.e., streptococci and leuconostocs	Not strictly a fermented milk but manufacture resembles that of cultured buttermilk; cream is inoculated and incubated until the desired acidity develops; flavor and aroma compounds are also contributed by the starter culture
Bulgarian milk	*Lactobacillus bulgaricus*	Incubation of inoculated milk at 37°C, but otherwise similar to cultured buttermilk; product differs from commercial buttermilk in having higher acidity and lacking aroma
Acidophilus milk	*L. acidophilus*	Milk for propagation of *L. acidophilus* and the bulk milk to be fermented is sterilized, since this organism is eaily overgrown by contaminating bacteria; incubation is at 37°C; acidity allowed to develop to 0.6 to 0.7%

Table 28-5. (continued)

Fermented Product	Principal Microorganisms Responsible for Fermentation	General Remarks
Yogurt	*Streptococcus thermophilus* *L. bulgaricus*	Made from milk in which solids are concentrated by evaporation of some water and addition of skim milk solids; product has consistency resembling custard; now common in Europe and North America; similar products with different names are produced elsewhere (see Fig. 28-7)
Kefir	*S. lactis* *L. bulgaricus* Lactose-fermenting yeasts	A mixed lactic acid and alcoholic fermentation; bacteria produce acid (0.6 to 1.0% lactic acid), and yeasts produce alcohol (0.5 to 1.0% ethanol); the organisms conglomerate to form small granules called kefir grains; the granules are used as the starter culture; in the Balkans, the fermentation is carried out in leather bags made of goatskin; the fermentation process may be continuous by adding fresh milk as the fermented product is removed; Kefir is made from cow, goat, or sheep milk
Kumiss	Similar to those found in kefir grains	A mixed acid-alcoholic fermentation product made from mares' milk in some parts of Russia

Table 28-6. Some Examples of Fermented Food Products

Fermented Food	Starting Product	Microorganisms Involved
Sauerkraut	Shredded cabbage	Early stage: *Enterobacter cloacae* *Erwinia herbicola* Intermediate stage: *Leuconostoc mesenteroides* Final stage: *Lactobacillus plantarum*
Pickles	Cucumbers	Early fermentation: *L. mesenteroides* *Streptococcus faecalis* *Pediococcus cerevisiae* Later fermentation: *Lactobacillus brevis* *L. plantarum*
Green olives	Olives	Early stage: *L. mesenteroides* Intermediate stage: *L. plantarum* *L. brevis* Final stage: *L. plantarum*
Sausage	Beef and pork	*Pediococcus cerevisiae* *Micrococcus* spp.

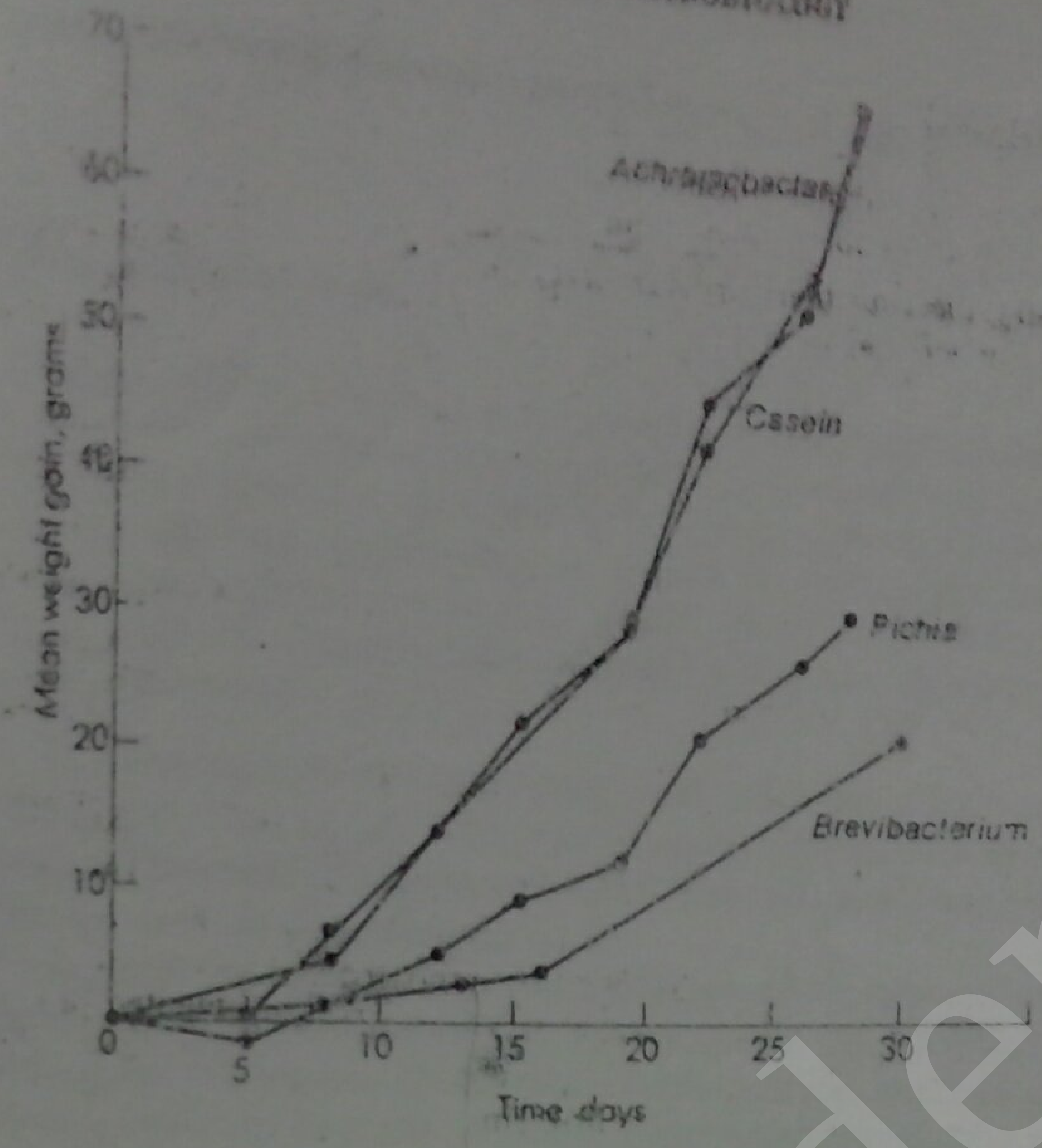

Figure 28-10. The nutritional quality of microbial proteins. This experiment shows the mean weight gain (per group) of rats fed various proteins: casein, bacterial proteins (*Achromobacter*, *Brevibacterium*), or yeast protein (*Pichia*). Note that the growth response to casein and *Achromobacter* protein was very similar. (Courtesy of V. F. Coty and R. I. Leavitt, *Dev Ind Microbiol*, 12, 1971.)

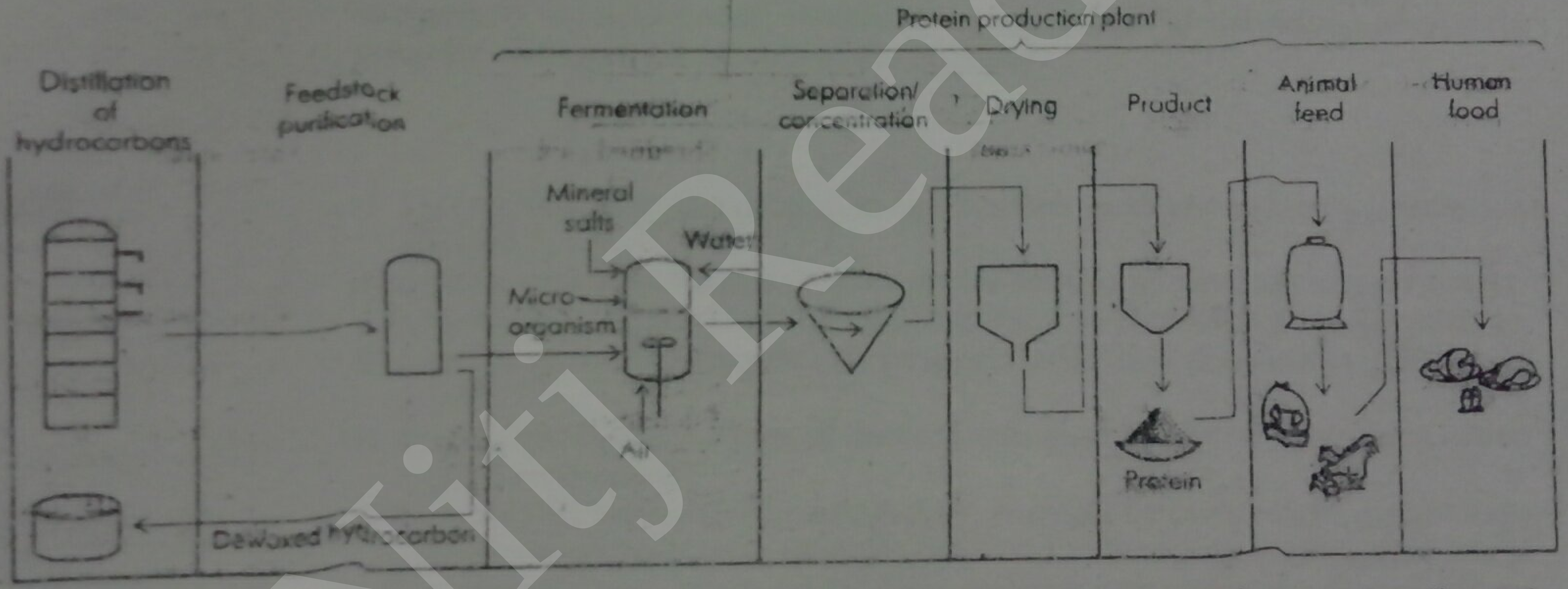

Figure 28-11. The process used by British Petroleum to produce single-cell protein from hydrocarbons. *n*-Alkanes are distilled for use in the fermenter. Minerals are added. Following fermentation, the cells are seperated and dried for use as animal feed. (Courtesy of British Petroleum Co. Ltd.)

This yield is 10 to 15 times higher than soybeans and 25 to 50 times higher than corn.

2 The protein content of the microbial cells is very high. Dried cells of *Pseudomonas* spp. grown on petroleum products have 69 percent protein; yeast cells have a protein content in a 40 to 50 percent range; for algae, the range is from 20 to 40 percent.

3 The proteins of selected microorganisms contain all the essential amino acids. An example of the nutritional quality of microbial proteins is shown in Fig. 28-10.
4 Some microorganisms, particularly yeasts, have a high vitamin content.
5 The medium (nutrients) for growth of microorganisms may contain industrial wastes or by-products, e.g., liquid paraffins (hydrocarbons) from oil refineries, spent sulfite liquors from the pulp and paper industry, beet molasses, and wood hydrolysates.

A fermentation system using yeast cells for single-cell protein production is shown in Fig. 28-11. The growth medium consists of hydrocarbons (*n*-alkanes) supplemented with mineral salts. The cell crop is harvested by centrifugation, dried, and used as animal feed.

Despite the very attractive features of single-cell protein as a nutrient for humans there are problems which deter its adoption on a global basis. For example, individual tastes and customs make microorganisms unattractive as a food substance to many persons. More specifically, the high nucleic acid content of microbial cells can produce intestinal disturbances. There is also the need to ascertain if the amino acid composition and content of the microbial protein meet the dietary requirements of the consumer.

QUESTIONS

1 List and describe the principles upon which methods of food preservation are based.
2 Compare the antimicrobial action of the following methods of food preservation: canning, refrigeration, dehydration, and increased osmotic pressure.
3 What is the lowest temperature range at which food-poisoning bacteria will grow?
4 What physiological types of bacteria are most likely to be present when canned food spoils?
5 Compare the types of microorganism that might be involved in the spoilage of refrigerated foods with those incriminated in the spoilage of canned foods.
6 List several types of microbial food spoilage, and name the organisms responsible in each instance.
7 Why is milk an excellent bacteriological culture medium?
8 Is milk sterile as it is drawn from the cow? Explain.
9 List the major sources of bacterial contamination of milk.
10 Describe the various types of biochemical changes brought about in milk by microorganisms. Identify the predominant types of bacteria responsible for each of these changes.
11 Of what particular significance are psychrophilic, thermoduric, and thermophilic bacteria in milk and milk products?
12 Is pasteurized milk sterile milk? Explain.
13 Compare the heat resistance of *Mycobacterium tuberculosis* and *Coxiella burnetii*. What bearing does this have on requirements for adequate pasteurization time and temperature?
14 What information does the phosphatase test reveal about milk?
15 What are the attractive features of food preservation through use of radiation?
16 Outline a procedure suitable for enumeration, isolation, and identification