Autoclave and Free-Flowing-Steam Systems for Heat Treatment of Infant Formula

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PROBLEMS engendered by the autoclaving of infant formula have been of concern to hospitals for many years. Because of the high temperature of the formula after autoclaving, many hospitals allow it to cool at room temperature for a considerable time before it is placed in the refrigerator. Further, preliminary surveys have indicated that about 24 hours of refrigeration is required to reduce the temperature of the formula to 44° F. A positive demonstration is needed of the validity of the flowing-steam (low temperature) heat treatment equipment provided with quick-cooling water methods, so that formula can be brought to storage temperature more rapidly in hospital refrigerators.

The autoclaving equipment currently used in hospitals entails the use of 230° F. steam under pressure for 10 minutes. This is a requirement in certain States. However, autoclaving at this temperature and time creates problems such as coagulation, nipple clogging, carmelization, nipple inversion, difficulty in cleaning nipples and bottles, and lack of adequate refrigerated space for cooling. Also, bacterial counts in samples taken from cooled formula may be due to the slow process of cooling it after autoclaving.

Commercially available free-flowing-steam equipment has been designed to eliminate many of the difficulties experienced by nurseries using the high-temperature, short-time autoclave method for heat treatment of infant formula. However, the flowing-steam method is not allowed in many States because of approximately 30-year-old regulations which had been enforced in an effort to control infant diarrhea. Consideration of a change in such regulations should be based on the results of thorough studies of available alternate methods (*1*).

Consequently, in the study reported here we proposed to install a free-flowing-steam unit and to compare bacteriological results in samples of formulas heat treated in this unit with similar samples heat treated in an autoclave.

Previous Studies

Bacteriological investigations of low-pressure and high-pressure techniques for terminal heating of infant formulas were reported in 1948 (*2, 3*). These studies revealed that the low-pressure technique (maintaining the temperature of the formula at 212° F. for 15 minutes) yielded complete destruction of selected species of vegetative cells inoculated into the formulas, but not of a spore-forming species. Sterility was determined by standard aerobic plate counts. The heat-penetration curves indicated a 7-minute bath come-up time (212° F.) and a 15-minute period to obtain 212° F. within the 8-ounce bottles. The high-pressure method (230° F. for 10 minutes) yielded only sterile samples, including those from formula inocu-

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lated with spore-forming species. To destroy common airborne contaminants in the formula, a temperature of 230° F. for at least 4 to 6 minutes was required.

Extensive research on the thermal destruction of microbes has been conducted and reported by food manufacturers and various private and governmental agencies. However, none of this research was specifically related to the problem engendered by hospital processing of infant formulas. The American Public Health Association has published the recommended procedures for sterilization of infant formula (*4*), but no direct comparison of commercially available equipment has been published which substantiates the effectiveness of the established standards for sterilization.

A recent hospital survey of the internal temperature and the bacterial counts of representative bottles of formula revealed that an excessively long time is required for cooling—24 hours to attain a temperature of 44° F. Additionally, bacterial counts were found in samples.

Study Design

An equipment manufacturer installed a low-temperature, free-flowing-steam unit at the University of Michigan School of Public Health so that a comparative study could be made of this unit and the high-temperature autoclave unit for heat treatment of infant formulas.

Since the University of Michigan Hospital prepares 11 different types of formula each day, we proposed that 11 samples be subjected daily to each type of heat treatment for a period of 3 weeks. This would be sufficient time to compensate for different days of the week, operational loads, personnel differences in preparation and distribution, and to test a large enough number of samples for statistical evaluation. The testing schedule for the 3-week period is shown in the table.

Methods

To compare the autoclave procedure (230° F. for 10 minutes) with the free-flowing-steam procedure (212° F. for 30 minutes), we undertook four separate phases of investigation: (*a*) study of the operating characteristics of the two systems, (*b*) comparison of the two systems in relation to viable micro-organisms, (*c*) comparison of the two systems using highly contaminated formulas, and (*d*) differentiating problems other than microbiological with bottled formula.

Operating characteristics. It was necessary to determine whether the heat-treatment equipment could attain and maintain the required temperature and time relationship for microbial destruction. Therefore, the first phase of this study was to determine the operating characteristics of the equipment by following the manufacturers' operating recommendations provided on the face plate and in printed instructions. Temperature was measured with six copper-constantan thermocouples attached to a calibrated multipoint recording potentiometer. The thermocouples were inserted at selected locations within the equipment and within representative bottles during the heat-treatment cycle, and the time required to attain and hold the prescribed temperature was also determined.

Viable micro-organisms. For this study, four extra bottles of each of the 11 formulas were prepared at the University of Michigan Hospital. The extra bottles were used for the following purposes: two bottles of each formula were heat treated in each system; one of the two was used for bacteriological testing and the other was used as a temperature indicator during a 24-hour holding period. The 24-hour period was used because it is the maximum period of use for any given formula. Temperature recordings were made only twice a week, and on days when temperature was not recorded two rather than four extra bottles were processed.

The following data were recorded for each experimental bottle of formula: the preparer or operator, date, time of heat treatment, system used (autoclave or flowing steam), the batch (if more than one batch of a particular formula was prepared), the load of the heat-treatment unit, and "for experimental purposes only."

The experimental bottles of formula were sent immediately to the school of public health where, on selected days, representative bottles were inoculated with predetermined numbers of spores of *Bacillus subtilis*, subjected to either of

Testing schedule for infant formulas and number of bottles positive for viable micro-organisms after specified type of heat treatment

Experiment No.[1] and testing procedure	Number of bottles	Number positive
3–31		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	5
4–1		
Bacteriological:		
Autoclave	11	1
Flowing steam	11	2
4–2		
Bacteriological:		
Autoclave	11	1
Flowing steam	11	1
Temperature, flowing steam	11	--------
4–3		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	4
Temperature, autoclave	11	--------
4–4		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	2
4–5		
Bacteriological:		
Autoclave	11	1
Flowing steam	11	3
4–6		
Bacillus subtilis: [2]		
Autoclave	11	0
Flowing steam	11	2
Temperature, flowing steam	11	--------
4–7		
Bacillus subtilis: [2]		
Autoclave	11	0
Flowing steam	11	2
Temperature, autoclave	11	--------
4–8		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	2
4–9		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	1
Temperature, autoclave	11	--------
4–10		
Bacteriological:		
Autoclave	11	1
Flowing steam	11	4
Temperature, flowing steam	11	--------
4–11		
Bacteriological:		
Autoclave	11	3
Flowing steam	11	4
4–12		
Bacteriological:		
Autoclave	11	1
Flowing steam	11	2
4–14		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	4
Temperature, flowing steam	11	--------
4–15		
Bacteriological:		
Autoclave	[3] 10	0
Flowing steam	11	2
4–16		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	1
Temperature, autoclave	11	--------
4–17		
Bacteriological:		
Autoclave	[3] 10	0
Flowing steam	11	4
Temperature, flowing steam	11	--------
4–18		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	2
4–19		
Bacteriological:		
Autoclave	11	0
Flowing steam	11	2

[1] Nos. 4–13 and 4–20 are described in text.

[2] Spores of *B. subtilis* were inoculated into formulas before heat treatment.

[3] 1 bottle broken during autoclaving.

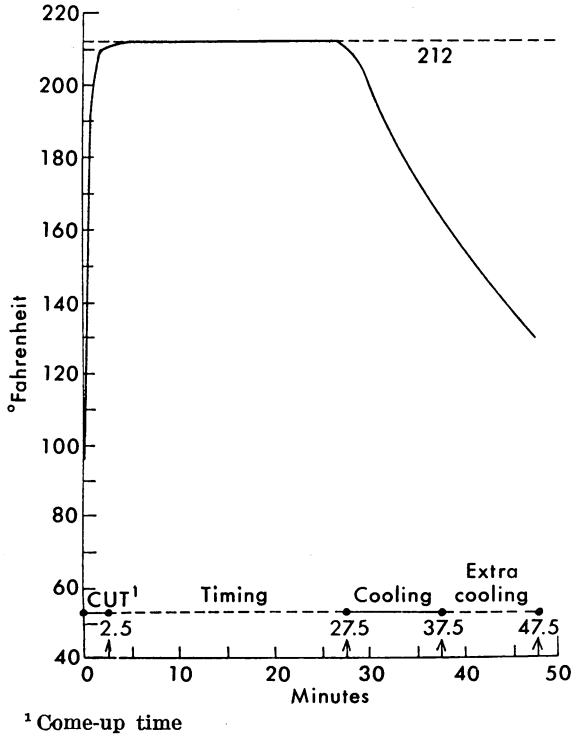
the two heat-treatment methods, cooled as recommended for each method, and refrigerated. After the 24-hour holding period the 22 experimental bottles of formula were tested bacteriologically to determine whether viable micro-organisms were present.

Bearing in mind that it is statistically possible for samples to contain viable microbes, we undertook this phase of the study to determine if the counts were significantly different between the samples heat treated in the autoclave and those heat treated in the flowing-steam system. During the test period, using 11 different formulas per day for 21 days, a total of 231 samples were heat treated in each system; however, two bottles were broken during autoclaving.

Highly contaminated formula. At the laboratory, the microbiological load of the formulas was increased by allowing those micro-organisms already present to increase in number by incubating the formulas at 98.6° F. for 4 hours before heat treatment.

For this phase two separate experiments were conducted on 2 test days. Five extra bottles of each of the 11 daily formulas were prepared at the hospital and sent to the laboratory. On delivery, one bottle of each formula was tested bacteriologically. A second group of 11 bottles (one of each formula) was designated for temperature measurement. However, since the potentiometer was equipped with six leads and one of these was required for measuring ambient temperature, five bottles were discarded and five of the remaining 10 contained thermocouples inserted through the nipples. The following summarizes this phase of investigation using 55 bottles of formula for each of the two experiments, Nos. 4–13 and 4–20 (in experiment 4–13 temperature was measured in the autoclave and in experiment 4–20 temperature was measured in the flowing-steam equipment).

Figure 1. Ambient temperature within the free-flowing-steam equipment



¹ Come-up time

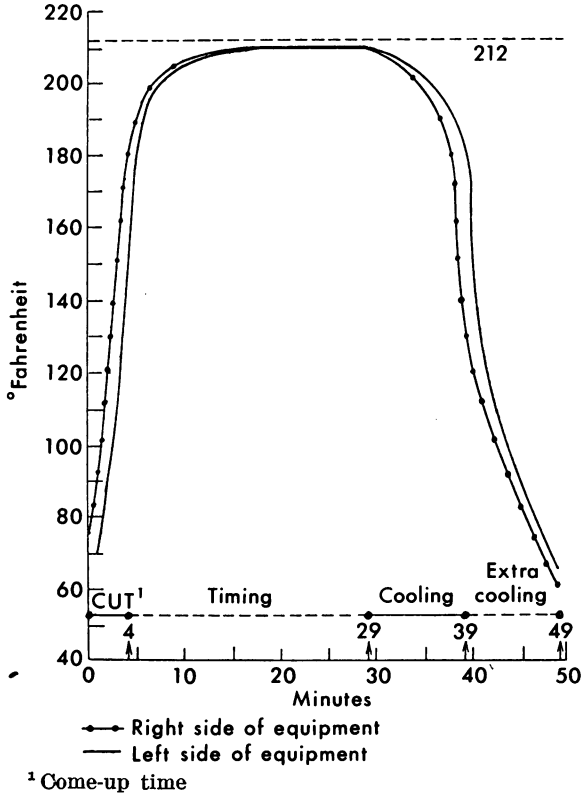
Testing schedule, experiments 4–13, 4–20	*Number of bottles*
Tested bacteriologically on delivery	11
Incubated 4 hours at 98.6° F. (37° C.)	44
Tested bacteriologically	11
Heat treated in autoclave and tested bacteriologically	11
Heat treated in free-flowing-steam unit and tested bacteriologically	11
Used as temperature indicators	5
Used to measure ambient temperature	1
Discarded	5

Problems other than microbiological. When the two systems were being compared in relation to viable micro-organisms, factors such as carmelization, nipple inversion, and operation difficulties were noted and recorded. These observations were necessary in order to evaluate the overall advantages and disadvantages of the two systems.

Results

Operating characteristics, The maximum ambient temperature within the flowing-steam equipment was 212° F. when the equipment recorder indicated 214° F. (fig. 1). The average temperature of water at the 4-ounce level (geometric center) in 8-ounce bottles is shown in figure 2. The dotted line shown for experiment 1 in figure 2 gives the average temperature of the water in five bottles located on the right side of the equipment. For experiment 2, the bottles were placed on the left side. The maximum average temperature attained was 209° F. for a period of 13 to 16 minutes.

Figure 2. Average water temperature at the 4-ounce level of 8-ounce bottles in various locations in the free-flowing-steam equipment



The water temperature of the flowing-steam unit was measured with a thermocouple, which was placed at the tip of the thermobulb in the equipment (fig. 3), and the recording device indicated a maximum temperature of 212° F. The automatic timer started when a temperature of 160° F. to 170° F. was attained. Little or no temperature variation occurred between bottles at a given time, except during the come-up period (up to about 5 minutes).

In experiments to determine if temperature stratification within the bottle was a factor, thermocouples were centrally placed at the 2-, 4-, and 6-ounce levels and in the air space below the nipple (figs. 4–7). The time required to reach maximum temperature was the same at the 2- and 4-ounce levels, and this time was longer than for the 6-ounce level or the airspace. Cooling was faster at the 2- and 4-ounce levels, because these are below the cooling water level.

Ambient temperatures within the autoclave, as measured by the potentiometer, ranged from 232° F. to 233° F. in different locations. The autoclave recorder showed 112° C. (234° F.) and the indicator pointed to 110° C. (230° F.).

The results of the study of the operating characteristics of the two heat-treatment systems indicated that (*a*) thermocouples should be located at the geometric center of the bottles (4-ounce level), (*b*) five thermocouples should be placed at the geometric center of different bottles and the sixth thermocouple should be used to measure ambient temperature, and (*c*) the locations of the bottles within the equipment will not affect the effectiveness of the heat treatment of formula.

Viable micro-organisms. Because a number of State health departments require that formulas be sterile, the bacteriological criterion for this study was practical sterility after 24 hours of holding at refrigeration temperature (45° F.). Sterility was tested by the methods described in Public Health Service Regulations-Biological Products (*5*), using fluid thioglycol-

Figure 3. Temperature at tip of thermobulb in free-flowing-steam equipment

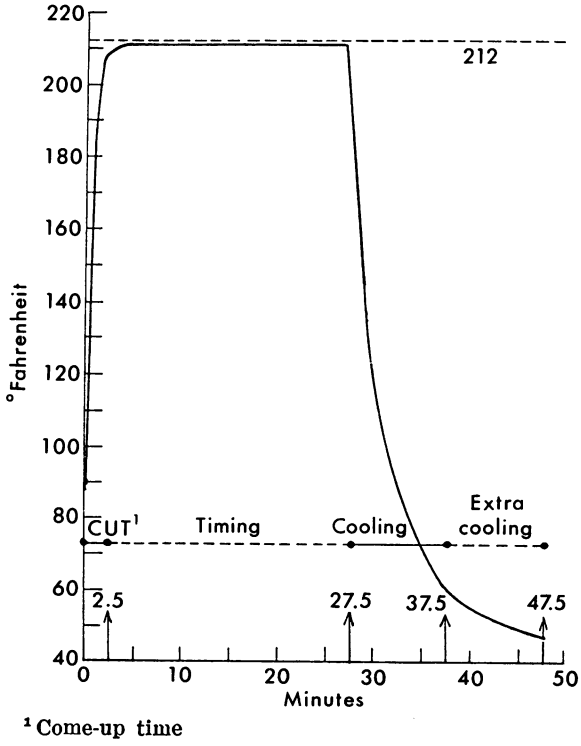
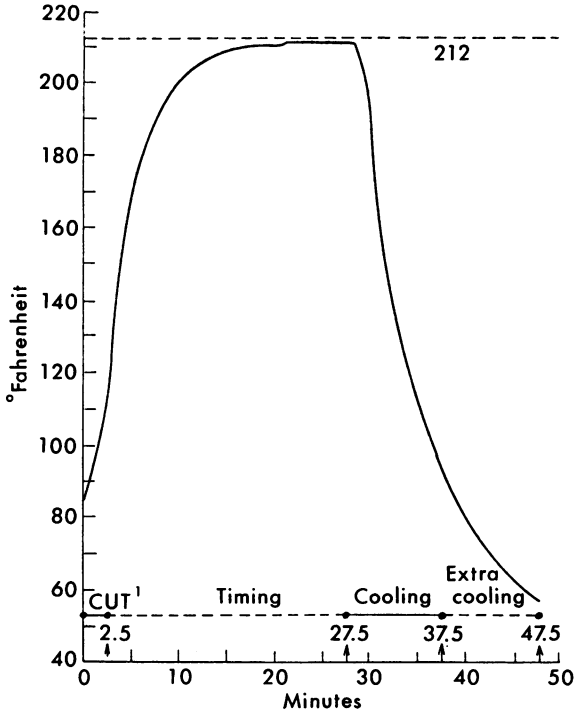
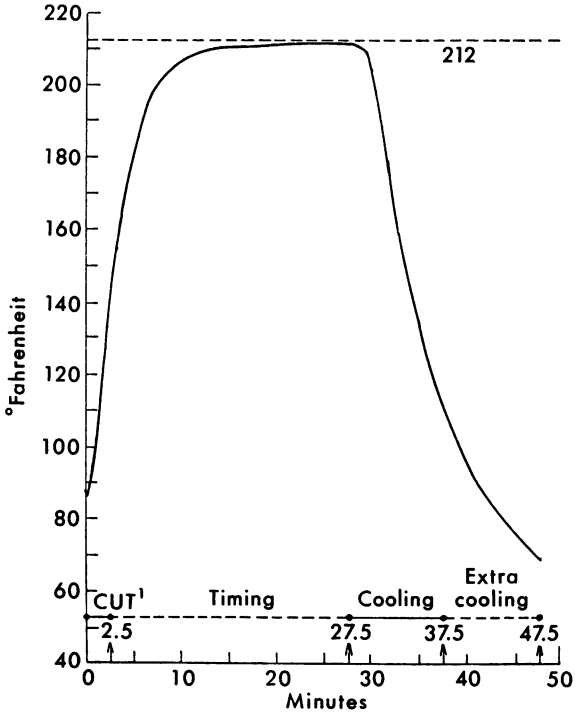


Figure 4. Water temperature at the 2-ounce level of 8-ounce bottles in free-flowing-steam equipment



[1] Come-up time

Figure 5. Water temperature at the 4-ounce level of 8-ounce bottles in free-flowing-steam equipment



[1] Come-up time

late medium. For each formula, 1 ml. of formula was pipetted aseptically into screwcap tubes containing 10 ml. sterile fluid thioglycollate medium. The tubes were incubated for 7 days at 86° F. Samples from the tubes which showed turbidity during this period were inoculated into fresh sterile media.

The number of positive formulas obtained with each of the heat-treatment systems is shown in the table.

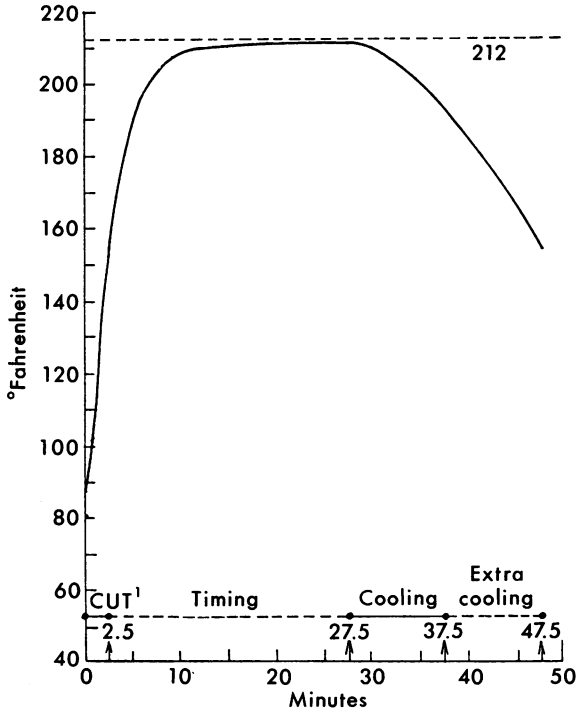
Statistical analysis, by the standard t test, of the results obtained with the flowing-steam equipment indicated that the average number of contaminated bottles (22.1 percent) was significantly greater than that which would be expected by chance ($P<0.1$). The contaminated bottles (3.5 percent) obtained when the autoclave was used could be attributed to chance ($P=4$) rather than to type of treatment. It is highly probable ($P=1$) that the differences observed between the two methods are significant.

As part of this phase of the study, spores of *Bacillus subtilis* were inoculated into formulas before heat treatment. The results are shown in the table (experiments 4–6 and 4–7). After autoclaving, none of the formulas were positive, but four of the 22 bottles heat treated with flowing steam were positive. Three of the four positive samples were due to gram-positive rods and the fourth was due to a chained gram-positive coccus.

Highly contaminated formulas. In experiments 4–13 and 4–20, the aerobic plate counts of formulas after incubation at 98.6° F. were increased twofold to 10^3-fold before heat treatment. After autoclaving, all the samples were sterile. The flowing-steam method, however, yielded one positive formula out of the 11 on each of the 2 test days. Each positive sample was from the same type of formula (Dryco) and was identified as a gram-positive rod. The aerobic plate count of the positive formula of experiment 4–13 was 9,700 organisms per ml. before heat treatment and for experiment 4–20 it was 620 organisms per ml.

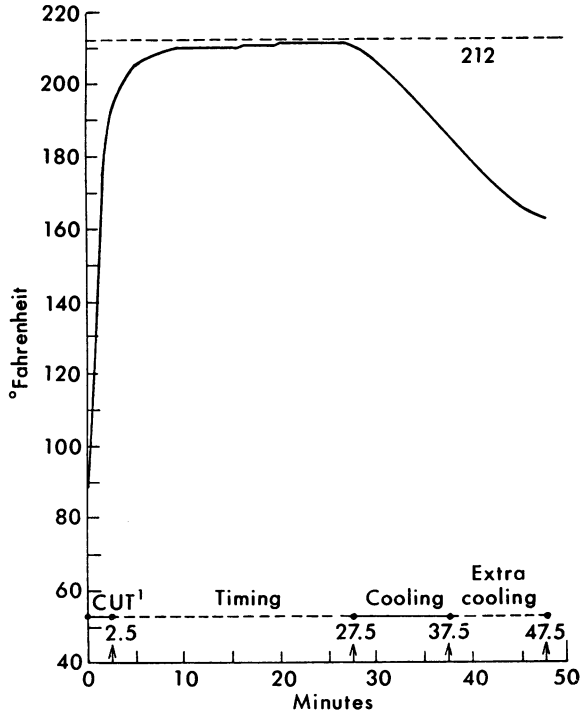
Problems other than microbiological. Various

Figure 6. Water temperature at the 6-ounce level of 8-ounce bottles in free-flowing-steam equipment



¹ Come-up time

Figure 7. Airspace temperature of bottles in free-flowing-steam equipment



¹ Come-up time

parameters, such as nipple inversion and carmelization, were of no significance in differentiating between the two heat-treatment systems in this study.

Temperature Studies

Temperatures were recorded within bottles during heat treatment and for 24 hours under refrigeration on certain days for each of the two heat-treatment systems. The slowest heating bottle for a given experiment was plotted. From these plottings, figures 8–13, it is apparent that the temperature at the geometric center of the formula did not attain the prescribed processing temperature in either of the two systems. With the autoclave, the maximum temperature attained was 227° F. for about 1 minute. With the flowing steam, the maximum temperature attained was 211° F. for 15 minutes, and four of the 11 samples in this experiment (4–17) were positive.

The maximum ambient temperature measured in the flowing-steam equipment was 211° F., and in the autoclave it was 233° F. (experiment 4–9). Generally, the temperature was 211° F. in flowing steam and 230° F. in the autoclave.

Conclusions and Recommendations

In designing the investigation, we considered many parameters necessary to evaluate the two heat-treatment systems for infant formula. The paramount criterion, however, was practical microbiological sterility of the formula by the recommended sterility test method. This criterion was not met by either system when the recommendations of the manufacturers or the State health department were followed. The results obtained with the free-flowing-steam method were significantly different from the sterility values expected by chance. Although some nonsterile formulas were obtained with the autoclave, they probably could be attributed to chance.

We believe that the positive samples occurred

because the necessary time-temperature relation within the formulas was not obtained. The flowing-steam method requires maintenance of the formula at 212° F. for 15 minutes and the autoclave method at 230° F. for 10 minutes. The temperature determinations indicated that these conditions were not met in this study. Of course, the time-temperature recommendations contain a safety factor. This factor was more realistic in the autoclave method, but it did not assure absolute sterility of the formula.

From the results of this study, it is not sufficient to set the timer and the indicator on the equipment and expect to obtain the required conditions unless the operating characteristics of the equipment are well known. Thus, peri-

Figures 8–13. Rate of heating and cooling of infant formulas in autoclave and free-flowing-steam equipment

Figure 8

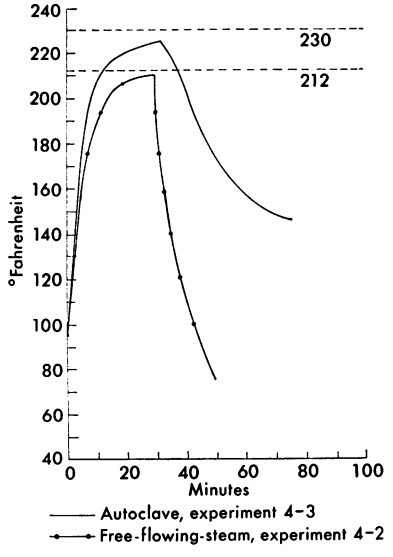


Figure 9

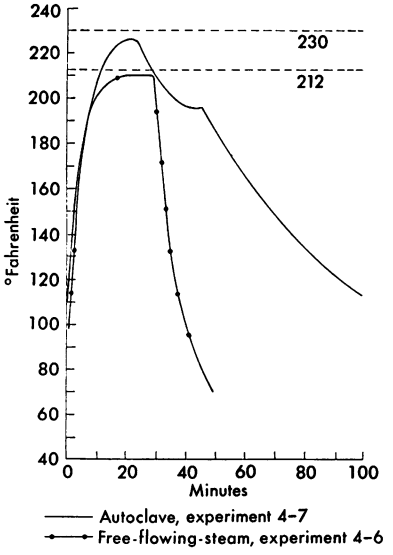


Figure 10

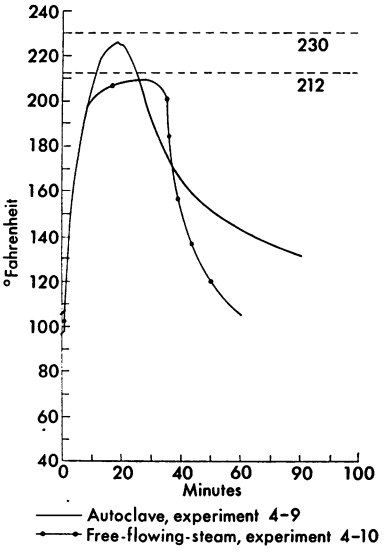


Figure 11

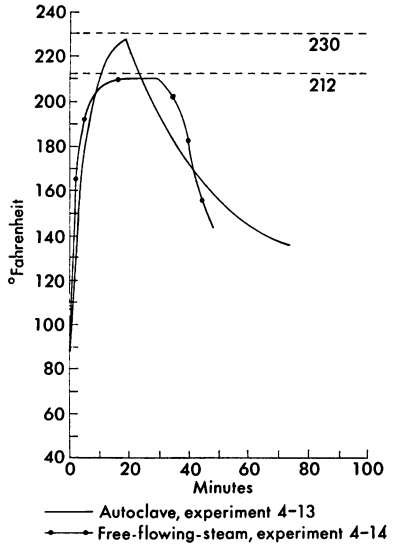


Figure 12

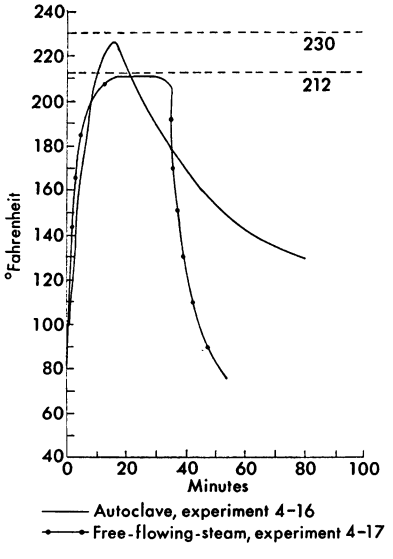
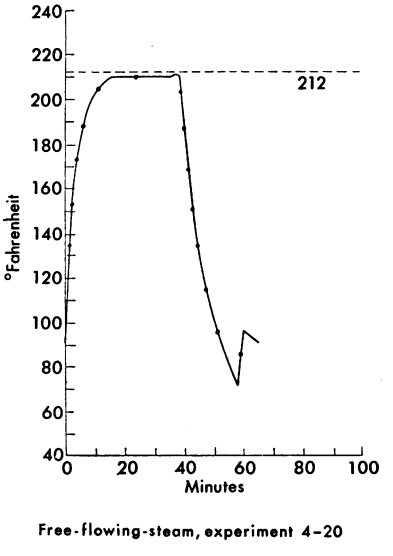


Figure 13



odic characterization of the equipment and calibration of the various quantitating devices are necessary.

Although the investigation did not indicate whether the recommended time-temperature relationships are valid, it did indicate that the recommended procedures may not yield the required result of sterility. Therefore, it is also necessary to review periodically the heat-treatment procedures and re-evaluate them in light of the calibration results.

When the formulas were contaminated with microbial spores or when the existing flora was increased, some positive formulas occurred with the flowing-steam method but not with the autoclave. As shown in figure 9, the spore-containing formula was maintained for 10 minutes at 210° F. in the free-flowing-steam unit whereas in the autoclave the formula was maintained at 225° F. for 6 minutes.

Parameters such as nipple inversion and carmelization were found to be of no significance in differentiating between the two systems.

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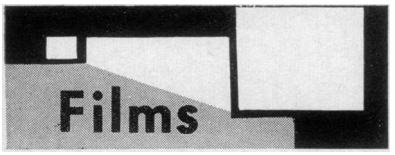
Three National Libraries to Coordinate Automation Efforts

The three national research libraries, National Agricultural Library, National Library of Medicine, and the Library of Congress, have agreed to adopt common goals as each proceeds to automate. This effort to achieve systems compatibility at the national level has far-reaching implications for library automation and library systems of the future. The purpose of the program is to improve access to the world's published material in all areas of human concern and scholarship.

Among the goals agreed upon by the three national research libraries are the development of a national data bank of machine-readable catalog information to be located in and serviced to other libraries by the Library of Congress, and a national data bank of machine-readable information relating to the location of hundreds of thousands of serial titles held by American research libraries. The latter would provide a computer-based system for scientists and scholars to use in locating the publications they need, anywhere in the United States.

A high priority in the cooperative program is the attainment of compatibility of the several authority lists of subject headings now used by the three libraries.

A task force has been named to identify problems and to make recommendations on cooperative programs. The members are Bella E. Shachtman, assistant director, Technical Services, National Agricultural Library; James P. Riley, chief, Technical Services Division, National Library of Medicine; and Stephen R. Salmon, executive officer, Processing Department, Library of Congress.



The Aedes Aegypti Inspector. *Motion picture, 16 mm., color, sound, 23 minutes, 1965. Order No. M–1151. Produced by the Public Health Audiovisual Facility for the National Communicable Disease Center, Atlanta, Ga.*

AUDIENCE: Primarily intended for *Aedes aegypti* inspector-spraymen and their supervisors.

SUMMARY: Shows techniques for making block and premises inspections for *Aedes aegypti* mosquito larvae. Demonstrates good public relations in contacting householders for approval to inspect premises, inspection equipment and its proper use in collecting larvae and adults, field recognition of mosquito larvae, and report preparation.

AVAILABLE: Free short-term loan from Public Health Service Audiovisual Facility, Atlanta, Ga. 30333, Attention: Distribution Unit. Purchase from DuArt Film Laboratories, Inc., 245 West 55th Street, New York, N.Y. 10019.

Laboratory Dogs. *Motion picture, 16 mm., black and white, sound, 17 minutes, 1966; cleared for television; order No. M–1446–X. Produced by Crawley Films, Ottawa, Canada, for the Animal Welfare Institute.*

AUDIENCE: Animal technicians, all schools of the health sciences, research foundations, hospital administrators, and veterinarians.

SUMMARY: Contrasts the life of a caged research dog with that of a group of compatible dogs that have undergone different types of experimental surgery at the modern animal laboratory at the University of Ottawa Faculty of Medicine. Includes such useful advice on animal care as stress on the importance of a clean, large room for the dogs to live in, daily exercise on a long roof runway, and the availability of enough food and water. Shows the postoperative care of a dog whose leg has been severed and replanted and the immediate postoperative care which includes the administration of pain-relieving drugs and fluids throughout the night. Later treatment and special feeding emphasize the importance of humanitarianism in research and experimentation with dogs.

AVAILABLE: Free short-term loan from Public Health Service Audiovisual Facility, Atlanta, Ga. 30333, Attention: Distribution Unit. Purchase from: Animal Welfare Institute, P.O. Box 3492, Grand Central Station, New York, N.Y. 10017.

Method for Rapid Electrophoresis. *Motion picture, 16 mm., color, sound, 11½ minutes, 1966; order No. M–1015. Produced by the Public Health Service Audiovisual Facility in collaboration with St. Joseph's Infirmary, Atlanta, Ga.*

AUDIENCE: Hospital technologists (trainees).

SUMMARY: Shows the electrophoretic apparatus, explains the functions of its parts, and how to set up the machine. Demonstrates a typical "run," explaining the technique of applying serum samples to the membrane and the step-by-step procedure of clearing and staining the resulting image.

AVAILABLE: Free short-term loan from Public Health Service Audiovisual Facility, Atlanta, Ga. 30333, Attention: Distribution Unit. Purchase from DuArt Film Laboratories, Inc., 245 West 55th Street, New York, N.Y. 10019.

Blood Collection for Pediatric Tests. *Motion picture, 16 mm., color, sound, 5 minutes, 1966. Order No. M–1299. Produced by the Public Health Service Audiovisual Facility for the National Communicable Disease Center, Atlanta, Ga.*

AUDIENCE: Practicing physicians and hospital personnel, especially nurses and laboratory technologists.

SUMMARY: Demonstrates the procedure for taking blood specimens from very young infants for phenylalanine testing and other pediatric serum tests. Shows faulty as well as proper techniques in using the Rasmussen disposable blood collector, with errors demonstrated and pointed out. Shows the recommended method for shipping the serum to the laboratory.

AVAILABLE: Free short-term loan from Public Health Service Audiovisual Facility, Atlanta, Ga. 30333, Attention: Distribution Unit. Purchase from: DuArt Film Laboratories, Inc., 245 West 55th Street, New York, N.Y. 10019.

The Health Fraud Racket. *Motion picture, 16 mm., color, sound, 28 minutes, 1967; cleared for worldwide television; order No. M–1426–X. Produced by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare.*

AUDIENCE: General public, movie theaters, television, industry, and older citizens' groups.

SUMMARY: Exposes the cunning traps and trappings of the fraud, the quack, and the charlatan who prey on all strata of society. In a series of colorful, fast-moving vignettes, tells how to distinguish between legitimate and fraudulent health services and products, and how to outwit the quack and quackery.

AVAILABLE: Free short-term loan from Public Health Service Audiovisual Facility, Atlanta, Ga. 30333, Attention: Distribution Unit. Purchase from: Capital Film Laboratories, 470 E Street SW., Washington, D.C. 20024.

Rapid Frozen Section Techniques. *Motion picture, 16 mm., color, sound, 5½ minutes, 1966; order No. M–998. Produced by the Public Health Service Audiovisual Facility in collaboration with St. Joseph's Infirmary, Atlanta, Ga.*

AUDIENCE: Hospital medical technologists (trainees).

SUMMARY: Demonstrates how the specimen is identified, trimmed for sectioning, placed on the microtome, and frozen. Subsequent scenes show how the frozen specimen is cut into thin sections, stained, and finally prepared for examination by the pathologist.

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