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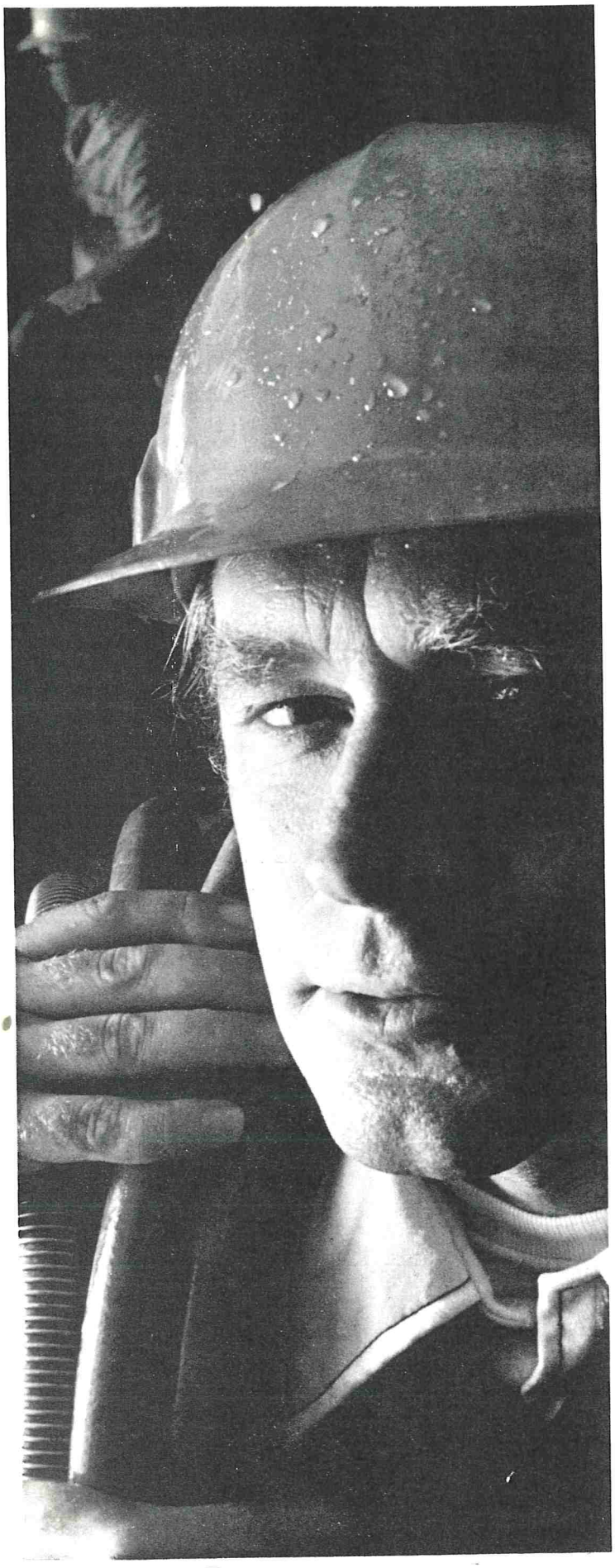

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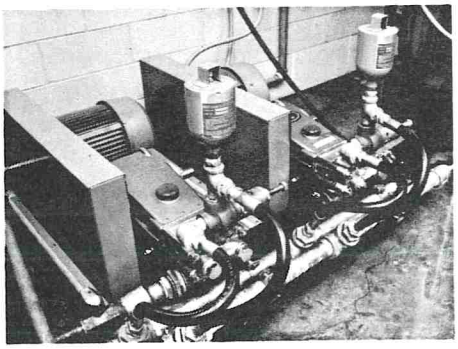

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PROFESSIONAL ACCOUNTABILITY IN ENVIRONMENTAL MANAGEMENT[1]

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(Received for publication March 25, 1974)

ABSTRACT

Professional accountability is an issue of high priority and great controversy in the environmental health field today. While it is an elusive concept, its terms of reference are in the broad spectrum of health, social, and economic issues either in the public or private sector. It includes a curious mixture of technical knowledge, and social and political responsibility all applied to a strategy designed to meet basic human needs and wants.

Of all the quests on which we are currently embarked, professional accountability is probably one of the most urgent. Indeed, this sense of urgency is clearly delineated in daily news media, in professional conferences, symposia, and among consumers of professional services of all types.

In the engineering field, Charles Yoder *(2)*, President of the American Society of Civil Engineers, devoted considerable attention to professional accountability in his 1973 President's Inaugural Address. Referring to the questionable practices of some professional civil engineers, he said: " . . . We cannot and will not tolerate bribes, kickbacks, corruption of public officials or payments to influence award of professional work."

Ludwig and Storrs *(1)* suggest that the engineer finds himself, at least in the United States, in a very pecular position "of having little creditability with the public in his own areas of expertise, including water quality control."

In the medical and pharmaceutical fields: A series of studies reported last February to the United States Senate Health Subcommittee showed that more than half the antibiotic drugs prescribed for hospital patients either are not needed or are prescribed incorrectly. These and related developments in the medical field prompted a three-column article in the February 25, 1974 edition of the *Wall Street Journal* entitled: "Do Doctors Need a Check Up?" The reporter asked: "Should a doctor, whose license to practice usually last for life, face relicensing? And should medical specialists prove they know the latest about brain surgery, broken bone repair, or cancer cures?"

For those of us on the "public payroll" there is continuing debate over the adequacy of our public service—food protection, solid waste collection, and a host of other governmental activities for which we are accountable.

In the face of spiraling costs, legislative bodies at all levels of government are pressing professional administrators to examine the long term consequences of their programs, and to find more palatable solutions than simply reducing services or increasing taxes. This charge within itself implies a need for a high level of professional accountability.

ACCOUNTABILITY ISSUES

But professional accountability cannot be achieved in a vacuum, and our capability to assume and maintain a high level of professional responsibility will depend upon how effectively and realistically we deal with some of the broader background issues that determine how effectively we promote professional growth and development in environmental management.

To be sure, every institution in society derives its basic purpose from the objectives of society as a whole. The total health and environmental enterprise in the United States is no exception. Therefore, to assess our reason for being a part of the community health and welfare team, we need to look first at society's reasons for supporting and encouraging our existence.

The first premise upon which American society is built is the intrinsic importance of the individual human being. In this we differ from nearly all other civilizations of the past and many of the present that have viewed the state as the ultimate object and the individual human being as a tool for its advancement. Our highest goal, therefore, that we share with other institutions of our society, is to protect the integrity and advance the fulfillment of the individual.

Because a healthful environment is a part of the foundation of individual self fulfillment, environmental health ranks among society's top priorities.

Everything we do in air pollution control, water

[1]Presented as the Keynote Address before the Michigan Environmental Health Association's Thirtieth Annual Conference on Environmental Health, Mt. Pleasant, Michigan, March 20, 1974.

resources management, food protection, and surveillance of ionizing radiation, all stem from this root. Society, recognizing environmental health as a condition of self-fulfillment, has set specific objectives for us.

It has been said that "the need for a healthful environment is common to all people, it cuts across boundaries of occupation, race, class, and politics . . . ."

Thus, every tax dollar earmarked and every private dollar spent for environmental health purposes testify to the importance of environmental management in the American scheme of things. It is not by accident that environmental health services cost in excess of 10 billion dollars annually. Yet, even a high level of environmental quality is, in essence, a means to a higher end rather than an end in itself. This is true both for the individual and for society.

Against that background the professional environmental manager will recognize that the convergence of new responsibilities for personal and community health services illuminate critical issues that transcend advances in engineering, the physical and biological sciences, or even the diminution of economic barriers.

These problems involve social and behavioral factors associated with the causation and distribution of disease, health practices, utilization of health services, and effectiveness of all services designed to protect and promote public health. It is here that the professional environmental health specialist must contribute to overall community health planning, not only at the descriptive level but even more at the level of program design.

Requirements of Accountability

Professional accountability requires that we acknowledge and fully accept the fact that the focus of community health planning is on the consumer. The consumer's needs, his priorities, and his effective participation in environmental health programs give direction and meaning to community health endeavors.

In this setting there is the continuing need for a communications bridge between consumers and health professionals. The practicing environmental health specialist, acting in the truest sense as consumer advocate, can help maintain this bridge because he enjoys the privilege, and more precisely, the responsibility not only for directly communicating with other agencies, and civic and social groups, but he is also required to deal directly with county, state, and federally elected legislative and administrative representatives; all of whom can influence policy decisions on health issues.

Professional accountability requires that we be good students, good listeners, and good readers on the problem of the environment that we must manage. We must be sensitive to what is happening in central cities if we are managing a suburban or rural environment and vice versa. This means an open mind and a tolerant attitude regarding the proposals and expressions of all groups, academic, ethnic, economic, labor, political, and youth who participate in the urban or suburban environment.

Responsible environmental managers must be great innovators. Such managers will be motivating mayors, councils, county commissioners, and community groups to creative actions in a wide variety of problem-solving exercises.

Such managers must perform as master technicians in meeting both physical and human problems because of their knowledge and experience. That is, we must supply key leadership in demonstrating the workability of techniques and proposals as yet far from proven for state or local government.

We must, if we are to be professionally responsible, lead successful community facilities programs, such as water treatment plants and improved housing, by assisting and advising political and other community leaders. In this assignment, we will need more than technical proficiency because significant decisions are hardly ever made by individuals but instead by a complicated process of brokerage, and bargaining of committee work and consensus. And so the expert at whatever level has the obligation not only to be right but also to be an effective salesman and organizer who can mobilize the work of others around his own perception.

Professional accountability mandates that we accept the fact that in some activities related to environmental health we will be in charge. In others we may be a member of a leadership team—sometimes a dominant member, sometimes a subordinate member. In still other situations we will not be included on the decision-making team at all unless we perceive the need for environmental health representation and work hard at including ourselves "in."

Sometimes the environmental health related issue will be raised by the people of the community or by action leaders whose responsibilities are tangential to environmental health. For example, the questions of intensive urban rat control, and the need for Congressional appropriations for such local services were not raised at first by environmental health specialists, but by "social welfare activists," who were later supported in their efforts by community health leaders. Sometimes we must be the one to identify the problem and raise the issue. In either instance we have a

mandate for responsible participation.

We will not always be welcome when we propose coordination of effort. Coordination is easy to favor in the abstract. But in practice one's reactions to coordination of services often depends upon who is coordinating whom.

Nor will we always be popular. As professionally responsible people and defenders of environmental quality we will sometimes appear to be defending against progress. How often have we been criticized for opposing more highway construction and supporting more mass transportation in our efforts to comply with the Clean Air Act of 1970?

THE SETTING FOR ACCOUNTABILITY

All of this can be summed up in a single sentence. Environmental health services at every level have entered the political and social arenas. The words "political" and "social" are used here in their broadest meanings.

There are many lions in those arenas. But they are where the actions are and where our professional accountability will be evaluated.

The basic decisions affecting environmental health are no longer made solely within the scientific and engineering microcosm. They are made at the highest level of social policy development and decision making. This is where the responsible environmental health worker belongs, as the spokesman for environmental health in public affairs.

But to function on that level we will need more than the traditional tools of the trade. More than ever before, professional accountability calls for courage. Not all new and worthy developments, no matter how important to the quality of the environment or to human health, will be acceptable to our colleagues, other elements of society, or the political hierarchy. Under these circumstances the professionally responsible administrator may have to make painful decisions and even face personal consequences. But a true professional must have confidence in the accuracy of his analysis of the problem, the wisdom of his course of action, and the overall good to society that will result from the program he proposes.

With the decline in epidemics because of improvements in the environment, and the willingness of the public and industry to accept sanitation as a way of life, we have much less need for police power and police methods. Today the need is for programs based on cooperative efforts of environmental health professionals, the general public, and industry. Futhermore, environmental health professionals must come to realize that the responsibility for promoting a safe and wholesome environment is not solely the responsibility of the environmental engineer or the environmental scientist.

Professional accountability dictates that we must now learn more than ever to shift from primary responsibility to shared responsibility and authority for environmental management and to develop new methods of collaboration and participation with nonprofessionals in the community.

To our benefit, the so-called nonprofessional environmentalists are no longer birdwatchers or little old ladies. They have become better organized and better informed and have in the main supported sound environmental programs, planning, and administration. Industry has also changed its attitude toward environmental health so radically and in so short a time that our distrust of the change is both apparent and understandable. But in the absence of supporting epidemic morbidity and mortality levels, environmental improvements must be promoted in terms of human wants.

Thus, the paramount question in the professional planning and administration of environmental health services is not so much what programs are being emphasized as it is what are the community's wants and are they given the necessary attention? There is a real tendency for environmental health administrators to continue to carry out programs developed some years ago while seldom, if ever, pausing to consider whether the program meets human wants or needs.

Professional accountability requires a redefinition of goals and a shift in resources required to meet those goals. For example, our traditional programs of "housing hygiene" must now be shifted to a concern for the full breadth and width of human settlements, including land use planning and development of higher standards for the total residential environment. This shift requires more than routine housing inspections and related code enforcement activities.

Too often in the past we have taken a very narrow view of problems that require public solutions. Results have been predictable: many problems have gone unsolved because of our failure to examine all their relevant dimensions. The system's approach offers a most useful strategy for overcoming this tunnel vision by probing the full magnitude of a problem to see it in the larger context with all its related elements.

We must view food protection in terms of the entire chain of events, from production on land or sea to the actual preparation and serving of meals and disposal of waste. Water quality must be viewed from the stages of planning, development, transportation, storage, treatment, distribution, and use on through to disposal or conservation and reclamation.

Professional accountability dictates that we argue

for a high quality and an effective level of program monitoring and evaluation partly because these are inputs into future policy and program development, but they also help to steer and guide the day-to-day management of programs and services. This process of in-course adjustment or *helmsmanship* recognizes that under the best of circumstances we will not always be able to anticipate all problems we encounter in the course of implementing a program, and that program development continues through program implementation, and at each significant point of implementation, professional accountability will be required and it will be put to the most severe tests.

In these situations it may be more productive to think about probabilities and recognize that in many environmental management areas, we cannot achieve 100% performance. In some programs 50 to 60% success may be a good justifiable result. Unfortunately, we have seldom faced this problem at the beginning of program planning because we were of the opinion that only 100% performance was a mark of high professional accountability.

It should be clear by now that professional accountability is not limited to keeping up with technological advances on one's own and related fields. This is only a beginning. The environmental manager must participate professionally in the social and political systems of his community. This is where the action is, where decisions are made, and where funds and other resources originate.

Environmental health professional societies and associations can no longer be dominant in determining environmental policy. We are only one bond in the spectrum of political, social, and health factors that the professionally responsible environmental health worker will consider before policy decisions are made.

But a professional environmental health association can help promote professional accountability. The association should lead in the constant reevaluation of its membership to make sure that it remains efficient, and follows a high code of ethics, and maintains honesty and integrity.

The association should provide programs of continuing education to lead the individual worker a greater understanding of disciplines other than his own. The association must prod the academic institutions to establish and maintain teaching and research programs that will benefit the practicing environmentalist. The professional association should use its certification and/or registration requirements to promote high quality environmental health services.

As far as professional training is concerned, admittedly it has a role to play, but we should remind ourselves that training which is not geared to promotion and career advancement is likely to contribute not to more effective utilization and professional accountability but to frustration and resignation. Why should a person undergo training unless he can profit thereby?

SUMMARY AND CONCLUSION

I fully recognize that environmental health workers, especially in state and local environmental agencies, grapple daily with restructuring, and redirection of effort and a host of other issues that impinge upon professional accountability. Not only are these activities of many types, but they are continually undergoing change, adaptation, and evolution. What is good and adequate today may be inadequate or outmoded tomorrow; an effective management system this morning may prove to be an ineffective approach to the same problem by late afternoon.

In contrast to most other private and public businesses there are relatively few activities in environmental health that are subject to formalization or formularization. In most instances the product to be sold tends to be intangible and the consumers are all people with various types and degrees of need, pride, prejudice, reticence, and intelligence.

Often the "selling" of environmental health literally involves a change in habits, desires, and customs of people, so that a successful approach in one instance may fail in other circumstances. Indeed, environmental management is a curious mixture of public and social responsibility and service — but so too is professional accountability.

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A Research Note

PRELIMINARY STUDY TO DETERMINE THE FEASIBILITY OF USING A 0.20-INCH DIAMETER DISK TO MEASURE SEDIMENT IN FLUID MILK

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ABSTRACT

A preliminary collaborative study was done to determine whether laboratory personnel could grade raw milk for sediment content using 0.20-inch diameter as well as 0.40-inch diameter sediment disks. The 0.40-inch disk presently is used for grading sediment in mixed bulk milk samples. Technicians in 10 separate laboratories made 800 determinations (80 per laboratory) of known sediment amounts on 0.20-inch and 0.40-inch diameter standard sediment disks; 225 (63.7%) of 400 determinations made on the 0.20-inch diameter disk were graded accurately, whereas 187 (46.8%) of the 400 determinations made on the 0.40-inch diameter sediment disks were graded correctly. Based on these results, it is recommended that photoprint standards be developed for 0.20-inch diameter sediment disks and that a further collaborative study be undertaken.

Sediment testing is one of the measurements used to determine the quality of fluid milk. When determined on milk in 10-gal cans, the procedure is known as an off-the bottom sediment test. In this test procedure a sediment gun, devised to reach the bottom of the can, draws up a one-pint sample of milk which immediately is discharged through a 1.25-inch diameter sediment disk. The amount of sediment remaining on this filtering disk is graded according to USDA standards *(3)*. As milk handling changed from cans to bulk milk tanks, this method became little-used.

In 1954, Liska and Calbert *(4)* developed a sediment test method for bulk tank milk using a one-pint mixed sample filtered through a 0.40-inch diameter disk. This method appeared in *Standard Methods for the Examination of Dairy Products (2)* and was accepted by regulatory authorities as an acceptable method for measuring sediment in a mixed sample of milk. However one-pint samples of milk from each producer are cumbersome for bulk milk haulers to handle properly. If the sample size were cut from one pint to 4 oz, this would facilitate handling. For this size sample to be used for sediment testing, the filtering diameter on the sediment disk needs to be reduced from 0.40 to 0.20 inch. Mechanically, this transition can be made easily. The purpose of this study was to determine the accuracy of using the 0.20-inch diameter disk to measure sediment in fluid milk.

MATERIALS AND METHODS

Ten laboratories from throughout the United States were selected to participate in this study. One technician in each laboratory was designated to make all the determinations. Personnel in all laboratories were familiar with sediment testing procedures, most performing sediment tests regularly.

Sediment disks were prepared with known amounts of sediment. They were prepared according to the fine sediment standard disk procedure *(1)*. Twenty samples were prepared in duplicate and sent to each laboratory for grading. One of the duplicates was graded using the 0.20-inch diameter disk and its duplicate was graded with the 0.40-inch disk. Technicians in each laboratory graded the sediment disks into four classes as recommended by the U. S. Department of Agriculture, Agriculture Marketing Service *(3)*.

The 0.40-inch diameter disks were graded by comparing them to standard photographs (grading charts) prepared by the U. S. Department of Agriculture in cooperation with the Food and Drug Administration and the American Public Health Association. Standard photographs for 0.20-inch diameter disks were not available; therefore, standards for 0.20-inch diameter disks were prepared by removing a 0.20-inch diameter disk from the center of the 0.40-inch standard disk photograph and overlaying it on an unused 0.40-inch diameter filter disk. All laboratories received the same instructions for grading the sample disks.

RESULTS AND DISCUSSION

Laboratory technicians determined sediment grade by indicating whether the sample disk belonged in Grade 1, 2, 3, or 4. Data from the laboratory technicians were evaluated as either being correct or incorrect. The analysis for this study was done by visual grading. Sample disks were number coded, identical numbers being used between laboratories. Grading results by laboratories are shown in Table 1.

Nine of 10 laboratories did a more accurate job

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TABLE 1. COMPARISON BY LABORATORIES OF ACCURACY IN GRADING 0.20- AND 0.40-INCH DIAMETER SEDIMENT DISKS

	0.20-inch diameter disk		0.40-inch diameter disk	
Lab number	Number correct determinations	Number incorrect determinations	Number correct determinations	Number incorrect determinations
1	26	14	12	28
2	24	16	20	20
3	20	20	13	27
4	23	17	27	13
5	24	16	18	22
6	28	12	19	21
7	28	12	26	14
8	20	20	11	29
9	33	7	28	12
10	29	11	13	27
Totals	255	145	187	213

TABLE 2. COMPARISON OF OVERALL LABORATORY GRADING ACCURACY

	0.20-inch diameter disk		0.40-inch diameter disk	
	Number	Percent	Number	Percent
Correct determinations	255	63.7	187	46.8
Incorrect determinations	145	36.3	213	53.2
	400	100.0	400	100.0

of grading the smaller (0.20-inch) diameter disk than they did the 0.40-inch disk. Laboratory No. 9 had the ability to grade samples correctly more frequently ($P < .05$) than the other laboratories. Laboratories were consistent in their ability to score the disks correctly, especially the smaller (0.20 inch) disks. When the larger disk (0.40 inch) was used, the laboratories showed considerable variation ($P < .05$) in their ability to correctly grade sediment on the disks.

An overall comparison of how laboratories graded the disks in shown in Table 2. These data illustrate that a more accurate ($P < .05$) determination was made on the smaller (0.20 inch) diameter disk than the larger (0.40 inch) disk.

Of the 400 determinations made by 10 laboratories on the 0.20-inch diameter disk, 255 (63.7%) were graded correctly. When these same laboratories graded for sediment using the 0.40-inch diameter disk, only 187 (46.8%) of the disks were graded correctly.

SUMMARY

This study points out that the 0.20-inch diameter disk can be graded more accurately than the 0.40-inch diameter disk. While a higher rate of inaccuracy than anticipated exists, less than 3.3% of the samples were graded incorrectly by two or more grades.

ACKNOWLEDGMENTS

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A SIMPLIFIED PROCEDURE FOR CONFIRMING SOMATIC CELL COUNTS OF MILK BY THE STRIP METHOD[1]

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Abstract

A practical method is described to reduce the number of strips counted in the confirmatory Direct Microscopic Somatic Cell Count (DMSCC) procedure. Using data from four commercial laboratories in widely differing geographic areas, and using a somatic cell standard of 1.5 million per milliliter of milk, the method was found to allow count discontinuance after the first strip from 47.3% of the time (where it was least effective) to 92.2% of the time (where it was most effective). Of 675 observations in the four sets of data, only six discrepancies were observed between results based on decisions made by this and the standard fixed-sample-size procedure.

The confirmatory Direct Microscopic Somatic Cell Count "strip" method requires the counting of four strips on two separate milk films. In a previous paper *(3)* a statistical technique was described that would allow counting to be discontinued, depending upon results obtained, after each individual strip. Preliminary data indicated that counts could be discontinued after the first strip in most instances, thus greatly reducing the labor involved in confirming somatic cell counts under the Abnormal Milk Control (AMC) program. Since other work *(4)* has shown that a considerable amount of laboratory work is done to uncover very small numbers of actionable-level counts, it was considered desirable to explore further this labor-reducing technique, and to establish, if possible, a practical method that could be used by laboratories confirming counts by the strip method.

In the work reported herein, data from four industry laboratories were combined and analyzed by several statistical approaches to determine the most practical procedures for reducing counting time by the "strip" method. While four laboratories may seem a relatively small number ,it should be pointed out that, to the best of our knowledge, very few laboratories are currently using the "strip" method for confirming counts. At the same time, the laboratories involved in this study did include widely different geographic locations and milk supplies—a sufficiently broad spectrum of milk supplies, the authors believe, to presume nationwide applicability of the technique described in this paper.

Materials and Methods

To broaden the data base over that reported earlier *(3)*, confirmatory somatic cell counts were obtained from three additional laboratories, all operating laboratories of Dairymen, Inc., one in Louisiana, another in Georgia, and a third in Virginia. These data were then combined with the Minnesota data for statistical evaluation. In all instances the data were collected during on-going operations of the laboratories involved. Only samples showing Wisconsin Mastitis Test (WMT) readings of over 21 mm (or 10% of those samples showing highest WMT reading down to but not including 21 mm) were confirmed, and all confirmatory testing was applied according to the procedure described in *Standard Methods for the Examination of Dairy Products (1)*. The sole divergence from regular procedure was to report the count on each individual strip separately, so that, aside from the average of the four strips counted on two films, data were available on each of the individual strips making up the four. Two strips, one horizontal and one vertical, were counted on each of two separate milk films. Strip factors for the four laboratories ranged from a low of 15,000 (two laboratories) to a high of 21,620. The strip factor in the fourth laboratory was 15,400. In total, 675 observations were made.

Once data were collected, several different statistical procedures were applied and evaluated for (*a*) comparability to the standard procedure and (*b*) efficiency in utilization of laboratory resources. For a discussion of the various techniques and the statistical theories on which they are based, the reader is referred to a companion paper by Bresson and Cook *(2)*. For this work suffice it to say that the statistic selected was a truncated sequential (likelihood ratio) procedure allowing for count discontinuance after each strip counted, and requiring no prior analysis of data in individual laboratories. This latter feature was considered particularly important for the possible adoption of the technique on a widespread basis. It should be pointed out, however, that it was not necessary to gain this advantage at the expense of other essential statistical considerations. The following, then, is a discussion of the practical implications and application of the Likelihood Ratio (LR) technique for counting somatic

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TABLE 1. SUMMARY CHARACTERISTITS OF SOMATIC CELL "STRIP" COUNT DATA SECURED FROM FOUR DIFFERENT COMMERCIAL LABORATORIES

Laboratory	Strip factor	No. of samples	Avg. no. of cells[1] (X_4)	As cell count/ml	Standard deviation (Sx)	As number of cells
1	15,000	207	471.0	(1.8×10^6)	121.4	(455,250)
2	21,620	115	289.7	(1.6×10^6)	92.0	(497,260)
3	15,000	122	353.9	(1.3×10^6)	122.1	(457,875)
4	15,400	231	228.6	(0.9×10^6)	85.1	(327,635)

[1]The X_4 count is the average number of somatic cells observed per sample, counting four strips on two milk films. All samples were previously screened by the Wisconsin Mastitis Test and only "confirmable" samples analyzed. To convert X_4 to a sample cell count divide the strip factor by four and multiply the X_4 by the resulting value. The same holds true for converting S_x to cell count.

cells under the microscope.

RESULTS AND DISCUSSION

Table 1 summarizes the characteristics of the data obtained on the four operating laboratories. Microscopic strip factors varied from 15,000 to 21,620. Somatic cell counts averaged, by the standard four-strip procedure, 1.8×10^6, 1.6×10^6, 1.3×10^6, 0.9×10^6/ml, respectively. Thus the data appear to represent a broad spectrum of potential counts and widely differing laboratory situations for which the LR technique should have application.

As an indication of the relative labor saving potential of the LR technique the percent of time somatic cell "strip" counts could be terminated after one strip was determined for each of the four laboratories. In the least desirable situation counting could be terminated after one strip 47.3% of the time. In the other three laboratories this percentage was observed to be 50.4, 66.4, and 92.2%, respectively. Overall this could result in substantial labor saving potential for the LR technique. The average number of strips that would have to be counted, for the four laboratories, respectively, were 2.0, 1.9, 1.6, and 1.1. In other words, for widely differing types of milk samples, counting would need to continue, on the average, for no more than two strips to get results essentially comparable to the four-strip standard procedure.

A comparison of the LR procedure with the standard procedure showed that, for 675 observations, final results would have differed in only six instances. This would appear to be a very small divergence from the standard and certainly well within expectations for a laboratory procedure of this kind. Reliability was also tested by determining the percentage of times a decision to stop counting would have been changed—at that precise count where such decision is most in doubt—if additional strips had in fact been counted. For a strip factor of 15,000 decision changes would have been made after the first, second, and

TABLE 2. STRIP FACTORS AND APPROPRIATE A1, B1 AND D VALUES FOR THE LIKELIHOOD RATIO METHOD OF COUNTING SOMATIC CELLS BY THE "STRIP" PROCEDURE

Strip factor	A1	B1	D
3000	168.5	211	187.3
3200	159.2	206.2	182.7
8400	155.1	201.5	178.3
8600	151.3	197.1	174.2
8800	147.6	192.9	170.2
9000	144.1	188.8	166.4
9200	140.7	184.9	162.8
9400	137.5	181.2	159.3
9600	134.4	177.6	156
9800	131.4	174.2	152.8
10000	128.6	170.9	149.8
10200	125.8	167.8	146.8
10400	123.2	164.7	144.
10600	120.7	161.8	141.3
10800	118.3	159	138.6
11000	116	156.3	136.1
11200	113.7	153.7	133.7
11400	111.5	151.1	131.3
11600	109.5	148.7	129.1
11800	107.4	146.3	126.9
12000	105.5	144	124.8
12200	103.6	141.8	122.7
12400	101.8	139.7	120.7
12600	100	137.6	118.8
12800	98.3	135.6	116.9
13000	96.7	133.6	115.1
13200	95.1	131.7	113.4
13400	93.5	129.9	111.7
13600	92.	128.1	110.1
13800	90.5	126.4	108.5
14000	89.1	124.7	106.9
14200	87.7	123	105.4
14400	86.4	121.4	103.9
14600	85.1	119.9	102.5
14800	83.8	118.4	101.1
15000	82.6	116.9	99.8
15200	81.4	115.5	98.4
15400	80.2	114.1	97.2
15600	79.1	112.7	95.9
15800	78.	111.4	94.7
16000	76.9	110.1	93.5
16200	75.9	108.8	92.3
16400	74.8	107.6	91.2
16600	73.8	106.4	90.1

16800	72.9	105.2	89
17000	71.9	104.1	88.
17200	71.	102.9	87.
17400	70.1	101.8	86.
17600	69.2	100.8	85.
17800	68.3	99.7	84
18000	67.5	98.7	83.1
18200	66.7	97.7	82.2
18400	65.9	96.7	81.3
18600	65.1	95.7	80.4
18800	64.3	94.8	79.5
19000	63.5	93.9	78.7
19200	62.8	92.9	77.9
19400	62.1	92.1	77.1
19600	61.4	91.2	76.8
19800	60.7	90.3	75.5
20000	60	89.5	74.8
20200	59.8	88.7	74
20400	58.7	87.9	73.3
20600	58	87.1	72.6
20800	57.4	86.3	71.9
21000	56.8	85.6	71.2
21200	56.2	84.3	70.5
21400	55.6	84.1	69.8
21600	55	83.4	69.2
21800	54.5	82.7	68.6
22000	53.9	82.	67.9

third strips, respectively, in 2.6, 3.7, and 1.7% of the samples. Again, this would seem to indicate very good reliability, certainly adequate for the needs of the AMC program.

To use the LR method, one simply selects the appropriate strip factor from Table 2, for example 15,000. Figures in colums A1 and B1 become the range of values used in counting the first strip. In all instances, when using the method, A1 is rounded up, B1 is rounded down. Thus, for a strip factor of 15,000 A1 = 83, B1 =*116*. If the count of the first strip exceeds 116, counting may be terminated; the standard has been breached. If the count falls at or between 83 and 116, counting continues to the second strip. To obtain the range of values used in strip two, the appropriate figure in column D (in this example 99.8) is added to A1 and B1, again rounding off as previously indicated. Therefore, strip two range is 82.6 + 99.8 = 182.4 (*183* rounding up) and 116.9 + 99.8 = 216.7 (*216* rounding down). Strip three values are 182.4 + 99.8 = 282.2 (*282*) and 216.7 + 99.8 = 316.5 *(316)*. For each strip the process indicated for strip one is repeated. If the B1 value is exceeded, counting is terminated. If the count falls at or between A1 and B1, counting continues. If, after the third strip, it is found that counting must continue, the fourth strip is counted and the somatic cell count determined as in the standard four-trip procedure.

Tabulating the foregoing, the A1 and B1 values used in the LR procedure for a strip factor of 15,000 are:

Strip no.	A1	B1
1	83	116
2	183	216
3	283	316

Once the above values are calculated, they stand, as rounded, and no further calculations are necessary.

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MICROBIAL PROFILE OF SELECTED SPICES AND HERBS AT IMPORT

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ABSTRACT

A partial microbial profile was determined for documented samples of imported and domestic spices. Bacteria of public health significance were absent. The incidence and numbers of amylolytic and proteolytic organisms, thermophilic sporeformers, yeasts and molds, and total microorganisms varied among the different spices as well as within each type of spice. When cells of *Salmonella* were inoculated in pre-enrichment cultures containing allspice, cassia, onion, and oregano, a definitive inhibition of growth was noted. To detect *Salmonella* in preparations of these spices, only small amounts of the condiment can be cultured.

Although spices are minor ingredients in foods, a substantial tonnage is used in the United States each year. It has long been known that spices may contribute significant numbers of microorganisms, including spoilage types, to a food product. However, aside from rudimentary numerical data, our knowledge of the microbial types that occur in spices is meager *(5)*.

Past studies have demonstrated that certain spices (e.g. black pepper) typically carry heavy loads of bacteria while others (e.g. cloves) have few. It has also been shown that sporeforming bacteria in spices may lead to spoilage of canned foods and processed-meat products and that the bacteria load can be reduced by ethylene oxide treatment *(3, 6)*. Little information is available, however, regarding the incidence and numbers of potentially harmful organisms such as salmonellae and staphylococci, and the so-called indicator organisms including *Escherichia coli* and the fecal streptococci. Moreover, the incidence of potential spoilage organisms including proteolytic types and thermophilic sporeformers has not been assessed in these products.

Since spices often come from areas of the world where sanitary practices are primitive, regulatory agencies are showing increasing concern over the microbiological content of these food ingredients. Our inadequate knowledge of the potential health problems associated with spices was the basis for this study.

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MATERIALS AND METHODS

Samples

Ten of the most widely used spices in domestic foods were chosen for this survey and each sample was identified as to geographical source, and date and port of entry into the United States. Some domestic samples were also examined. The majority of the spice samples were procured by professional samplers at the port of entry. Generally, 0.5 to 1.0 kg of spice, taken by a "thief" sampler, were placed in plastic bags and submitted to the laboratory for analysis. Upon receipt, samples were stored in the cold (4 C) until analyses were undertaken (holding did not exceed a two-week period).

Before sampling for microbiological analysis, the spice was mixed manually in the plastic bag in an effort to insure homogeneity. Ten grams of spice were blended for 1 min wth 90 ml of 0.1% peptone water contaning 0.1% Tergitol-7 in a sterile, mechanical blender. With leafy spices, such as oregano, only 1 g was used in 99 ml of diluent.

Total count

Additional decimal dilutions were made in peptone water from the initial blend. Duplicate pour plates were prepared using Plate Count Agar (PCA, Difco). After the agar had solidified, plates were capped with PCA to limit growth of molds and spreaders. Plates were incubated at 30 C for 48 h before being counted. Colonies were picked from each of the plates into Brain Heart Infusion (BHI) broth (Difco) for indentification.

Yeast and mold count

Dilutions prepared for total plate counts were also used for making spread plates on Potato Dextrose Agar (Difco). These plates were incubated at 23 C for 96 h before counting.

Spore count

A sterile, screw-cap tube containing 10 ml of the initial blend was immersed in a water bath at 70 C for 10 min, then removed and rapidly cooled in an ice bath. Further decimal dilutions were prepared and plated. Plates were poured and capped with PCA followed by incubation for 48 h at 30 C.

In addition to the pour plates, a three-tube most probable number (MPN) determination was done to estimate the thermophilic anaerobe population. Decimal dilutions of the heat-shocked suspension were used to inoculate tubes of Brewer's Thioglycolate Medium (Difco). These tubes were tempered in a water bath at 55 C, then incubated 48-96 h under anaerobic conditions at 55 C. Any turbidity in the thioglycolate cultures constituted a positive result.

Proteolytic count

Proteolytic organisms were estimated by making pour plates with Frazier's gelatin agar *(2)* using the appropriate dilutions from the initial blend. After 48 h at 30 C, plates were flooded with a solution of acidified mercuric chloride and the proteolytic numbers were estimated.

Amylolytic count

Amylolytic organisms were counted by making pour plates with starch agar (Tryptone, 10 g; yeast extract, 5 g; glucose, 0.5; K_2HPO_4, 2.0; NaCl, 5g; soluble starch (Difco), 3.0; agar, 15 g; distilled water, 1liter; pH 7.0-7.2) using the appropriate dilutions from the initial blend. After 48 h at 30 C, plates were flooded with an iodine solution.

Other determinations

Standard methods *(1)* were used for *Salmonella, Staphylococcus aureus,* and *Escherichia coli* estimations. To determine whether a spice or herb was toxic to *Salmonella,* a lyophilized mixture of *Salmonella ohio, Salmonella newport,* and *Salmonella typhimurium* was added to each of 35 spices at a level of approximately one *Salmonella* per 10 g of spice. In most instances, the spices were pre-enriched in nutrient broth using the standard concentration of 10% (w/v). When light leafy spices were pre-enriched, a 5.0-7.5% concentration was used to allow for the increased volume of the light material. If *Salmonella* could not be detected when pre-enriched at a level of 10%, this ratio was reduced until the toxicity of the spice was diluted enough to allow the organisms to grow.

RESULTS

To facilitate presentation of data, results obtained for each of the 10 spices and herbs are discussed individually. However, for comparative purposes, the results concerning the various populations that were estimated (i.e. total plate count, spore count, etc.) are grouped and presented in tabular form.

In addition to routine counts, three physiological types of bacteria were quantitated in each sample: *(a)* thermophilic organisms that can grow under anaerobes conditions (i.e., true anaerobes and facultative anaerobes such as *Bacillus stearothermophilus* and *Bacillus coagulans*) *(b)* proteolytic organisms; and *(c)* amylolytic organisms.

Black pepper

Twelve samples of black pepper from seven sources were analyzed. Six samples originated in Indonesia, three in Brazil, and three in India. Characteristically, black pepper has been associated with high counts of aerobic sporeformers and the samples we examined were no exception (Tables 1 and 2). All samples had total counts and spore counts in excess of one million organisms/g. Two of the samples from Brazil contained low numbers of *S. aureus* and *Aerobacter.* Two other samples, that originated from different continents and entered through different ports, contained low numbers of enterococci. No *Salmonella* were detected.

TABLE 1. TOTAL PLATE COUNTS OF IMPORTED AND DOMESTIC SPICES

Range of counts (Organisms/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia[1]	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤1,000	0	1	0	0	0	1	1	0	0	0	0
≤10,000 >1,000	0	3	0	1	3	5	8	3	0	0	1
≤50,000 >10,000	0	2	0	0	1	1	1	5	3	0	3
≤100,000 >50,000	0	1	1	2	2	1	0	4	5	0	1
≤200,000 >100,000	0	2	3	0	0	0	0	1	3	0	5
≤300,000 >200,000	0	0	0	0	0	0	0	0	0	0	0
≤500,000 >300,000	0	0	2	0	0	0	0	0	1	0	0
≤1,000,000 >500,000	0	1	2	0	0	1	0	0	2	0	0
≤10,000,000 >1,000,000	2	0	2	3	0	0	0	0	2	9	0
≤50,000,000 >10,000,000	6	1	0	0	0	0	0	0	0	1	0
>50,000,000	4	0	0	0	0	0	0	0	0	0	0
Total no.	12	11	10	6	6	9	10	13	16	10	10

[1]One sample was uncountable due to mold growth.

TABLE 2. BACTERIAL SPORE COUNTS OF DOMESTIC AND IMPORTED SPICES

Range of counts (Spores/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤1,000	0	2	0	0	0	3	2	2	0	0	0
≤10,000 >1,000	0	5	0	2	2	5	8	3	1	0	7
≤50,000 >10,000	0	3	0	1	2	1	0	6	3	0	3
≤100,000 >50,000	0	0	3	1	2	0	0	2	4	0	0
≤200,000 >100,000	0	1	1	0	0	0	0	0	3	0	0
≤300,000 >200,000	0	0	1	0	0	0	0	0	2	0	0
≤500,000 >300,000	0	0	2	0	0	0	0	0	1	0	0
≤1,000,000 >500,000	0	0	1	0	0	0	0	0	0	0	0
≤10,000,000 >1,000,000	2	1	2	2	0	0	0	0	2	9	0
≤50,000,000 >10,000,000	6	0	0	0	0	0	0	0	0	1	0
>50,000,000	4	0	0	0	0	0	0	0	0	0	0
Total no.	12	12	10	6	6	9	10	13	16	10	10

TABLE 3. YEAST AND MOLD COUNTS OF DOMESTIC AND IMPORTED SPICES

Range of counts (Organisms/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤10	6	6	10	4	6	9	9	10	14	9	5
≤1,000	1	1	0	0	0	0	1	2	2	1	0
>10											
≤10,000	2	2	0	0	0	0	0	1	0	0	3
>1,000											
≤50,000	1	0	0	2	0	0	0	0	0	0	1
>10,000											
≤100,000	1	1	0	0	0	0	0	0	0	0	0
>50,000											
≤200,000	1	1	0	0	0	0	0	0	0	0	0
>100,000											
≤500,000	0	1	0	0	0	0	0	0	0	0	0
>200,000											
>500,000	0	0	0	0	0	0	0	0	0	0	0
Total no.	12	12	10	6	6	9	10	13	16	10	9

TABLE 4. THERMOPHILIC ANAEROBE COUNTS OF DOMESTIC AND IMPORTED SPICES

Range of counts (organisms/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤10	0	6	2	4	2	6	8	7	2	3	7
≤1,000	4	6	4	1	3	3	1	5	2	2	2
>10											
≤10,000	0	0	3	1	1	0	1	1	12	1	1
>1,000											
≤50,000	8	0	1	0	0	0	0	0	0	4	0
>10,000											
Total no.	12	12	10	6	6	9	10	13	16	10	10

Cassia

Twelve samples of raw cassia were submitted from seven sources. Nine originated in Indonesia, two in the Seychelles, and one in Madagascar. The counts varied widely (Tables 1-6), showing a thousand-fold difference in almost all categories. Although there was a wide numerical variation, most samples had total counts less than 200,000/g, spore courts of less than 50,000/g, and all had very low counts of thermophilic anaerobes. The number of amyloytic and proteolytic organisms approximated the spore count. The sporadic incidence of molds is probably a consequence of physical factors such as storage and handling. No *Salmonella, S. aureus,* or any indicator organisms were found. One Indonesian sample contained a few *Aerobacter* sp.

Celery seed

Ten celery seed samples from six sources were submitted. Nine of the samples originated in India while the tenth came from France. The flora of the samples consisted mainly of proteolytic and amylolytic sporeformers (Tables 1, 5, and 6). One of the Indian samples contained a significant number of *Aerobacter*, whereas enterococci were found in another Indian sample. All samples were free of mold (Table 3), but they varied widely in numbers of thermophilic anaerobes (Table 4). There were no *Salmonella, S. aureus,* or *E. coli.*

Ginger

Six samples of ginger were obtained from five sources. Four of the samples originated in Nigeria, and one each in Sierra Leone and Jamaica. In half of the samples, total counts exceeded one million/g, while in the remainder less than 100,000/g were detected (Table 1). Both the Sierra Leone sample and one of the Nigerian samples had large populations of sporeformers (Table 2). The Jamaican sample had a large population of micrococci. Whether these variances reflect different harvesting techniques cannot be ascertained with the limited number of samples. No *Salmanella, S. aureus* or indicator organisms were found.

Mace

The mace samples were in good microbiological condition with the flora consisting almost exclusively of proteolytic and amylolytic sporeformers (Tables 1, 5, and 6). Most of the samples contained some

TABLE 5. COUNTS OF PROTEOLYTIC ORGANISMS IN DOMESTIC AND IMPORTED SPICES

Range of counts (Organisms/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤1,000	0	1	1	2	0	4	4	5	0	0	4
≤10,000	0	5	0	0	2	2	4	5	1	0	3
>1,000											
≤50,000	0	2	2	2	4	1	2	3	6	0	2
>10,000											
≤100,000	1	1	1	0	0	2	0	0	4	0	0
>50,000											
≤300,000	0	3	3	0	0	0	0	0	1	0	1
>100,000											
≤500,000	1	0	2	0	0	0	0	0	1	0	0
>300,000											
≤1,000,000	0	0	0	0	0	0	0	0	0	0	0
>500,000											
≤10,000,000	3	0	1	1	0	0	0	0	0	8	0
>1,000,000											
≤50,000,000	5	0	0	1	0	0	0	0	0	2	0
>10,000,000											
>50,000,000	2	0	0	0	0	0	0	0	0	0	0
Total no.	12	12	10	6	6	9	10	13	13	10	10

TABLE 6. COUNTS OF AMYOLYTIC ORGANISMS IN DOMESTIC AND IMPORTED SPICES

Range of counts (Organisms/g)	Spice examined (No. of samples in stated range)										
	Black pepper	Cassia	Celery seed	Ginger	Mace	Mustard seed	Nutmeg	Oregano	Paprika domestic	Paprika imported	Rosemary
≤1,000	0	3	0	1	0	3	3	4	0	0	5
≤10,000	0	3	1	1	4	4	7	5	1	0	4
>1,000 <50,000	1	2	2	1	2	2	0	4	7	0	1
≥10,000 ≤100,000	1	1	1	1	0	0	0	0	3	0	0
>50,000 ≤300,000	0	2	5	0	0	0	0	0	2	1	0
>100,000 ≤500,000	0	1	1	1	0	0	0	0	1	1	0
>300,000 ≤1,000,000	0	0	0	0	0	0	0	0	2	1	0
>500,000 ≤10,000,000	4	0	0	1	0	0	0	0	0	7	0
>1,000,000 ≤50,000,000	5	0	0	0	0	0	0	0	0	0	0
>10,000,000 >50,000,000	1	0	0	0	0	0	0	0	0	0	0
Total no.	12	12	10	6	6	9	10	13	16	10	10

thermophiles. No *Salmonella*, S. *aureus*, or indicator organisms were detected.

Mustard seed

Nine samples of mustard seed were submitted from five sources. Three originated in Canada and six came from domestic sources. The samples were similar in count except for one, which had a high total count and an unusually high gram-negative population (Table 1). The latter proved to be a mixture of *Proteus* and *Alcaligenes*. Apparently some gram-negative organisms can survive for considerable periods in certain types of spices. Although one sample did have a high number of organisms, the others had total counts of less than 100,000/g (Table 1). All samples had spore counts and amylolytic counts (Tables 2 and 6) of less than 50,000/g, while the majority had proteolytic counts of less than 50,000/g. No molds, *Salmonella*, S. *aureus*, or indicator organisms were found. The number of thermophilic anaerobes was generally low (Table 4).

Nutmeg

Ten samples of nutmeg were submitted from six sources. Four samples originated in Indonesia, three in the East Indies, two in the West Indies, and one in East India. All of the nutmeg samples contained very low microbial loads (Table 1). Some variations were observed in that one sample from Indonesia contained a small population of micrococci and one sample each from East India and Indonesia contained thermophiles (Table 4). No *Salmonella*, S. *aureus*, or indicator organisms were detected.

Oregano

Thirteen samples of oregano were submitted from eight sources. Ten samples originated in Greece while the remaining three were imported from Mexico. The oregano samples did not vary widely either in count or in types of organisms. All plate counts were less than 200,000/g (Table 1); no physiological types numbered more than 100,000/g (Tables 2-6). No *Salmonella*, S. *aureus*, or indicator organisms were found.

Paprika

As with black papper, high aerobic spore counts are common in paprika. Domestic paprika was not as heavily contaminated as its imported counterpart. Most of the samples contained thermophiles and three of the Spanish paprikas had significant numbers of enterococci. No *Salmonella*, S. *aureus*, or *E. coli* were detected.

Rosemary

All samples contained less than 200,000 bacteria/g. Thermophilic bacterial counts were low; mold counts were variable; no *Salmonella* were detected.

A comparison of the microbial profiles for the spices and herbs examined in this study is presented in Table 7 with the means and ranges of the counts. This table affords not only an estimation of variability within each type of condiment but it also presents an overall summary of the results.

Effect of spices on Salmonella recovery

To determine whether any spice is toxic to the growth of *Salmonella*, 25 g of each sample were added to 225 ml of nutrient broth. A lyophilized mixture of *Salmonella* species (see Materials and Methods) was rehydrated and diluted so that each sample received 2 or 3 *Salmonella* cells (as determined previously by plate counts). Each *Salmonella* test was done in duplicate on three separate occasions; 35 spices were employed in this series of tests (Table 8).

Viable *Salmonella* cells were not recovered from allspice, cassia, oregano, or granulated onion using the standard AOAC 10% pre-enrichment procedure. By reducing the ratio of sample to pre-enrichment broth to 5.0%,*Salmonella* cells were detected in allspice and granulated onion. To recover *Salmonella* consistently from oregano and cassia, it was necessary to reduce the spice/pre-enrichment broth ratio to 0.5% while maintaining the 2 or 3 cell inoculum/225 ml of broth. Previous investigators have noted the inhibitory effect of onion on the growth of *Salmonella* (4).

TABLE 7. MICROBIAL PROFILE OF SELECTED IMPORTED AND DOMESTIC SPICES (COUNT/g OF SPICE)

Spice (No. samples in brackets)	Mean (M) and range (R) of counts	Standard plate count	Bacterial spore count	Thermophilic anaerobes	Proteolytic organisms	Amylolytic organisms	Yeasts and molds
Black pepper (12)	M–	32,000,000	32,000,000	20,000	20,000,000	850,000	19,000
	R–	5,500,000 to 50 million	5,500,000 to 50 million	500 to 30,000	75,000 to 50 million	30,000 to 50 million	10 to 150,000
Cassia (12)	M–	280,000	480,000	250	51,000	71,000	53,000
	R–	1,000 to 30 million	1,000 to 5,500,000	10 to 500	1,000 to 150,000	1,000 to 400,000	10 to 350,000
Celery seed (10)	M–	150,000	1,310,000	4,800	680,000	120,000	10
	R–	75,000 to 750,000	75,000 to 750,000	10 to 30,000	1,000 to 5,500,000	5,500 to 400,000	0 to 9
Ginger (6)	M–	2,700,000	1,800,000	1,000	5,900,000	100,000	10,000
	R–	5,500 to 5,500,000	5,500 to 5,500,000	10 to 5,500	1,000 to 30 million	1,000 to 5,500,000	10 to 30,000
Mace (6)	M–	32,000	36,000	1,100	21,000	13,000	1
	R–	5,500 to 75,000	5,500 to 75,000	10 to 5,500	5,500 to 30,000	5,500 to 30,000	0 to 9
Mustard seed (9)	M–	98,000	6,700	170	21,000	9,400	1
	R–	1,000 to 750,000	1,000 to 30,000	10 to 505	1,000 to 75,000	1,000 to 30,000	0 to 9
Nutmeg (10)	M–	7,500	4,600	600	8,600	4,100	49
	R–	1,000 to 30,000	1,000 to 5,500	10 to 5,500	1,000 to 30,000	1,000 to 5,500	10 to 505
Oregano (13)	M–	47,000	26,000	600	9,400	11,000	1,200
	R–	5,500 to 150,000	1,000 to 75,000	10 to 5,500	1,000 to 30,000	1,000 to 30,000	10 to 5,000
Paprika-domestic (16)	M–	860,000	790,000	4,100	79,000	160,000	77
	R–	30,000 to 5,500,000	5,500 to 5,500,000	10 to 5,500	5,500 to 400,000	5,500 to 750,000	10 to 505
Parika-imported (10)	M–	4,900,000	7,900,000	24,000	10,000,000	3,900,000	59
	R–	5.5 to 30 million	5.5 to 30 million	10 to 30,000	5.5 to 30 million	150,000 to 5,500,000	10 to 505
Rosemary (10)	M–	92,000	12,800	650	23,000	5,700	5,100
	R–	550 to 150,000	5,500 to 30,000	10 to 5,500	1,000 to 150,000	1,000 to 30,000	10 to 30,000

DISCUSSION

In previous microbiological studies of spices, no particular attention was given to documentation of samples. They were obtained usually from grocery stores, restaurants, or distributors. In this study an attempt was made to determine the origin, port of entry, date of entry, and other significant data concerning handling and quality of the spices. Since all of the history was given for most of the spices, certain generalizations can be made:

(*a*) There was no apparent correlation between the country of origin and the quality of spice with respect to numbers of microorganisms or predominant flora. Thus, a country that produces a spice of high quality with respect to flavor does not necessarily produce a microbiologically superior product.

(*b*) No organisms posing a public health hazard were found.

(*c*) The sporadic occurrence of some species of bacteria such as micrococci, enterococci, and reportedly *E. coli* and *Salmonella* may also be expected since thousands of small growers with diverse growing and harvesting techniques are involved in many of the spice-producing countries. However, our results indicate that these occurrences are relatively rare.

Certain trends were noted in the study. Generally, samples containing high total counts also contained high numbers of proteolytic, amylolytic, and thermophilic bacteria. Mold counts did not correlate with total counts and the contamination and/or growth of these organisms in the spice samples must be totally independent. Indications of fecal contamination (*E. coli counts*) were absent. This may be due to the susceptability of *E. coli* to desiccation.

The absence of *Salmonella* in all the original samples reflects the low incidence and sporadic nature of contamination with these organisms. However, in the current study, 113 samples were examined, and none was found to be contaminated with *Salmonella*.

The apparent toxicity of cassia, oregano, allspice, and onion to *Salmonella* reduces the likelihood of detecting this pathogen in these spices. Paprika presents a minor problem in that it contains a rather high

TABLE 8. RECOVERY OF INOCULATED *Salmonella* FROM PREENRICHMENT CULTURES CONTAINING VARIOUS CONDIMENTS[1]

	Amount of condiment in preenrichment culture (wt/vol)			
Condiment tested[2]	10%	7.5%	5.0%	0.5%
Allspice	–	–	+	0[3]
Bay leaves[4]	0	0	+	0
Cassia	–	–	–	+
Celery flakes[4]	0	+	0	0
Chives, freeze-dried[4]	0	0	+	0
Marjoram[4]	0	+	0	0
Onion, granulated	–	–	+	0
Oregano	–	–	–	+
Parsley flakes[4]	0	0	+	0

[1]See text for explanation of inoculum, its level and the methodology involved.

[2]Inoculated *Salmonella* were detected in preenrichment cultures containing 10% of the following condiments: anise, red bell peppers, cardamon, celery seed, cut chillies, chili powder, comino (cumin), coriander, curry powder, dill seed, dill weed, cleaned fenugreek, ginger, mace, dry Chilean mushroom, mustard seed, nutmeg, paprika, black pepper, white pepper, rosemary, sage, sesame seed, spearmint, tarragon, vegetable flakes.

[3]Not tested.

[4]Lower preenrichment ratios of spice: broth were required with leafy condiments.

content of carbohydrate. It is suggested that a buffered pre-enrichment broth be used with this spice to preclude a rapid pH drop brought about by competing organisms.

In toto, the results of this investigation gave no indication of a potential health problem associated with spices and herbs. It would appear that spices, like other food ingredients, may in rare instances become contaminated with microorganisms of public health significance. However, multiplication in these dried products is precluded, and longevity of vegetative cells in the dry state appears to be limited.

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PREVENTION OF FOODBORNE ILLNESS BY TIME-TEMPERATURE CONTROL OF THAWING, COOKING, CHILLING, AND REHEATING TURKEYS IN SCHOOL LUNCH KITCHENS[1]

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ABSTRACT

Turkey meat served in schools is often incriminated as a vehicle of foodborne illness. Preparation practices that could contribute to outbreaks were studied in three schools. *Clostridium perfringens, Staphylococcus aureus,* and *Salmonella* were isolated from raw turkey carcasses. *C. perfringens* was also isolated from cooked turkey meat and from food-contact surfaces of equipment. Twenty-pound carcasses that were thawed either in a refrigerator, or in paper bags at room temperature, showed no significant opportunities for multiplication of bacteria; carcasses thawed at room temperature without bags could, however, have supported multiplication of psychrotrophic bacteria. The several days required for refrigerator-thawing is, unfortunately, more time than can always be provided in school food service schedules. All methods of baking were satisfactory. Turkey rolls took more time to reach an internal temperature of 165 F than is practicable for school food service operations. Less than half an hour was saved by baking half instead of whole turkeys. Refrigerated whole turkeys and pots of stock cooled slowly. Immersing double-plastic-bagged turkey meat and halved turkey rolls in an ice bath and slicing turkey meat onto ice-cold pans speeded cooling. Stock was chilled rapidly when half-filled five-gallon pots were set in either an ice or a running-water bath. Chilled turkey meat was reheated to 165 F in steamers, in kettles of boiling gravy, in lid-covered pans on a range, and in ovens. The ovens were slowest to bring the meat to the required temperature—often too slow to be practicable. Twenty ways to reduce risk inherent in thawing, cooking, chilling, and reheating practices are described.

Reports from health agencies to the Center for Disease Control indicate that turkey meat is frequently incriminated as a vehicle for foodborne illness. It was responsible for 13% of all reported foodborne disease outbreaks in which specific vehicles were identified for the period 1961-1970 *(32, 34)*. During these years, turkey meat and turkey salads or dressing were responsible for 11% of 425 outbreaks of staphylococcal intoxication. Since the beginning in 1962 of the Center's surveillance program for *Salmonella,* turkey meat has been the vehicle of 23% of 171 foodborne outbreaks of salmonellosis *(33)*. Cooked turkey meat has also been the vehicle of 21% of 124 confirmed outbreaks of *Clostridium perfringens* foodborne illness that were reported from 1966 through 1970 *(34)*.

Schools and institutions where large quantities of foods are prepared frequently are places where turkeys have been mishandled in such a way that outbreaks ensued. From 1966 through 1970, for instance, 143 foodborne outbreaks—11% of the total reported—occurred in schools and accounted for 35,843 cases—39% of the total.

These national data reflect situations in States. In Georgia, for example, 104 outbreaks have been investigated from 1961 to 1970. Of these outbreaks, 22 occurred in elementary and high schools, and 13 that resulted in 2,939 cases were associated with serving turkeys.

Turkeys are incriminated in foodborne outbreaks for three reasons. In the first place, raw turkeys are often contaminated with *Salmonella (8, 9), Staphylococcus aureus (18), and C. perfringens (10)* when they are received in kitchens. Inadequate cooking may allow these bacteria to survive. Secondly, turkeys require considerable handling, such as deboning and slicing, before they are served. Cooked turkeys are handled in the same general area as the raw ones, by the same personnel who previously handled the raw ones, and sometimes on or in the same equipment used for the raw ones. This handling not only increases the chances for recontamination with *Salmonella* from raw turkeys, it also increases the risks of contaminating the product with S. *aureus* or *C. perfringens* from human sources. Thirdly, and perhaps most important, turkeys are almost always prepared at least a day before serving. In this amount of time, foodborne pathogens that got into the turkeys during handling or those that survived cooking can multiply in the meat or stock if the foods are not cooled rapidly to 45 F or less and held at that temperature.

Unfortunately, cooling is seldom accomplished

[1]Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department Health, Education and Welfare or the Georgia Department of Human Resources.
[2]Center for Disease Control.
[3]Georgia Department of Human Resources.

rapidly enough. Turkey meat and stock cool slowly when refrigerated in a conventional manner, and, very often, school kitchens do not have enough refrigerator space to store the number of turkeys needed to serve the students. Large pots and pans are frequently used for refrigerator storage of the meat and stock. Sometimes, turkeys are even left overnight in ovens which are warm but have been turned off. Both practices provide ideal conditions for bacterial growth. On the day of serving, turkey meat, gravy, or dressing are often not heated or are only warmed to a temperature less than 165 F, or hot gravy is often poured over cold meat. Such practices will kill neither cells of *C. perfringens* that have emerged from spores nor any post-cooking contaminants that have multiplied during storage. These and other factors that contribute to foodborne outbreaks are reviewed in greater detail by Bryan *(7)*.

With these contributory factors in mind, bacteriological and time-temperature surveys were made to evaluate turkey-handling practices at three school kitchens. The studies were made to find some practical ways of preventing or at least minimizing the factors that contribute to outbreaks which sometimes follow the serving of turkeys in schools.

METHODS

Time-temperature evaluations. Various types of premium grade, type T[2] thermocouples were used to determine temperatures of turkey and stock during thawing, cooking, chilling, and reheating. Thermocouples with stainless steel bayonet sensors 4 to 8 inches long were inserted into the center of the thickest part of the turkey breasts, into the geometric center of turkey rolls, and into the center of pieces of meat. As much as possible of the metal shaft was buried in the meat. Flat, button-type thermocouples were used to take surface temperatures. These were held in place by two wires wrapped around the thermocouple wire near the sensor so that each end extended in a different direction. Rubber bands were hooked to one end of fastening wires, brought around the turkey, and hooked to the opposite extending wires. Tension of the rubber bands secured the thermocouple button to the skin surface. Thermocoples with smooth, plastic wire coverings and blunt-end metal sensors were used to test midpoints of stock and gravy. Thermocouples with plain, welded ends were used to obtain temperatures of the air (room, oven, refrigerator, freezer) that surrounded the turkeys being tested. Data were recorded on a chart in a potentiometer[2].

Frozen turkeys, weighing 20 lb. or more, were thawed in their original plastic wrapper on tables at room temperature, in double-layer kraft paper bags at room temperatures, and in a forced-air, reach-in refrigerator. Boxes containing four turkeys were removed from a freezer and held at room temperature 1-1/2 h before temperature recording began. A heated ice pick was used to make a hole for thermocouple insertion into the breast. A bayonet-type thermocouple was then inserted into the hole and pushed into the center of the thickest part of the breast. For each turkey that was tested, a skin surface temperature was also taken. When turkeys were thawed in paper bags, thermocouple-wired turkeys were put into the bags, and the open ends were folded and taped. Thermocouples that were used to take room, paper bag, and refrigerator air temperatures were located a few inches from the turkeys being tested.

Turkeys were prepared for cooking in several ways. Turkey rolls were prepared in the school kitchens by first skinning a turkey. Meat was cut from the bones, then rolled and wrapped with skin. Ends of the skin were then sewed together and strings were tied around the roll. Turkeys that were to be roasted in halves were sawed in half through the center of the breast bone. All turkeys and rolls were seasoned with salt, pepper, and butter, and approximately 1 quart of water was put into roasting pans.

Thawed turkeys and rolls were cooked at various temperatures in conventional gas ovens (range and shelf-type), in forced-convection electric ovens, and in steam kettles. A foil tent was used to cover turkeys while roasting in conventional ovens. During cooking, thermocouples were inserted into the center of the thickest part of the breast or into the geometric center of rolls, secured to the surface of the skin of the breast or rolls, and placed in oven or kettle so as to be within a few inches of the turkeys. Whole turkeys, turkey halves, and turkey rolls were roasted in ovens, and eight whole ones were cooked in a steam kettle filled with water.

After the turkeys were cooked, they were cooled at room temperature for 1 to 3 h and then put into a refrigerator. Some turkeys while warm were deboned and cut into bite-size pieces; 8-1/2 lb. of these pieces were put into 19-1/2 × 11-1/4 × 2-1/2-inch pans. Whole turkeys were also stored overnight in a walk-in refrigerator. For experimental purposes, a half turkey was left overnight in an oven that had been turned off.

Cut-up turkey meat was chilled rapidly by putting portions of it in direct contact with pans placed in larger pans of ice, into pans placed in refrigerators or freezers, and into double plastic bags submerged in crushed ice.

A turkey roll was cut in half. One half was wrapped in aluminum foil and refrigerated. The other half was put into double plastic bags, put in a pan, covered with ice, and then refrigerated. The pieces of meat used for temperature measurements were cut into rectangles of approximately 1 × 1 × 4 inches, which exceeded the average size of the cut-up meat. A 4-inch, bayonet-type thermocouple was inserted into the center of one end of the specially cut pieces of meat and pushed lengthwise three-fourths of the way through the piece.

Methods of rapidly cooling heated (160 F) turkey stock were evaluated by cooling 2.4 gal in a 5-gal stockpot (9-1/2 inches in diameter × 11 inches deep). Cooling methods consisted of putting the pot into a pan of ice, into a sink continuously filled with running water to a level that exceeded the level of stock in the pot, into a walk-in refrigerator (50 F), or on a table at room temperature. Still another method consisted of pouring an equal amount of heated (160 F) stock into a mixer pan and agitating in a vertical mixer for 30 min at low speed 1 and for an additional 30 min at speed 2. The water level in the sink, that was used as the water bath, was regulated by cracking the outlet valve and by allowing a pencil stream of water to flow into the sink. During each cooling experiment, the stock was stirred at 5-min intervals except when stock was mixed or when it was stored in the refrigerator.

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[1]Type T refers to copper-constantan junction.

[2]Temperatures were recorded on Chart 5270 (type T), 0 to 500 F range, in an Electronik 16 Multipoint Recorder, Honeywell, Fort Washington, Pa.

Each experiment lasted for 1 h, except when the stock was refrigerated, it was cooled overnight.

Stock that was left after turkeys were cooked in the steam kettle remained in the kettle for a few hours and then was ladled into a 14-gal stockpot. This pot was filled with 12.4 gal of stock, carted into a walk-in refrigerator, and stored overnight.

Chilled, cooked turkeys and stock were reheated in several ways on the day they were served to students. Gravy was prepared from stock, flour, butter, and seasonings, and heated to boiling in a stockpot on a range or in a steam kettle. Whole turkeys that had been refrigerated overnight were deboned and cut into small pieces. This meat was put into pans and heated in a steamer or left at room temperature for up to 1-1/2 h. Both heated and unheated pieces of meat were put into boiling gravy in the steam kettle and cooked. Cold, cut-up pieces of meat were also put into pans, hot gravy was poured over the meat, and it was then heated in ovens or on a range. Temperature recordings were made of the center of the gravy and of the inside of 1 × 1 × 4-inch pieces of meat during the entire reheating process. Turkey with gravy or turkey and gravy over rice were served from a steam table immediately after these heat treatments.

Dressing was baked in 2-1/2-inch deep pans in ovens on the day the turkeys were served. Temperature recordings were made in the geometric center of the dressing and in the oven about an inch above the top surface of the dressing.

Sampling and laboratory analysis

Four swabs were simultaneously rubbed over thawed, raw turkeys and food-contact surfaces of equipment. Two of the swabs were put into tubes containing cooked meat dextrose broth or thioglycolate broth (BBL), one into tetrathionate brilliant-green broth (Difco), and one into brain heart infusion broth (Fisher). Samples of spices, stock, and cooked turkey were aseptically put into sterile plastic bags. These samples were refrigerated until cultured (within 24 h).[4]

One tube of either cooked meat dextrose broth or thioglycolate broth was heated to 80 C for 10 min; the other tube was not heated. Tubes of cooked meat broth were then incubated anaerobically at 37 C for 24 h; tubes of thioglycolate were incubated at 45 C for 5 to 6 h. The incubated, cooked meat broth was streaked to modified McClung Toabe egg yolk agar. The incubated thioglycolate broth tubes that showed gas were streaked onto sheep blood agar plates. Suspicious colonies were picked and confirmed or ruled out as *C. perfringens* by gram staining, inoculation into indole-nitrite broth (BBL), litmus milk (Difco), and motility medium (Difco), and streaking onto egg yolk agar (colonies from blood agar plates). Twenty grams of shredded cooked turkey were added to 100 ml cooked meat dextrose broth. Stock was shaken and then 1 ml was added to each of two tubes of 10 ml cooked meat dextrose broth. These samples were then examined as described for the swab samples.

Tetrathionate broth tubes were incubated at 37 C overnight and then streaked to brilliant green agar plates. After 18 to 24 h incubation at 37 C, suspicious colonies were picked to triple sugar iron agar slants. These slants were incubated overnight at 37 C. Suspect cultures were confirmed or rejected as *Salmonella* by urease testing and slide agglutination. Twenty milliliters of stock were added to 200 ml tetrathionate broth containing brilliant green and turgitol. Fifty grams of turkey meat were added to 450 ml sterile phosphate buffered distilled water in a blender; the blender was operated for 2 min, 1 min at low speed and 1 min at high speed. Twenty milliliters of the homogenate were added to 200 ml of tetrathionate brilliant green turgitol broth. The stock and meat cultures were examined as described for the swab samples.

Brain heart infusion broth tubes were incubated overnight at 37 C. The incubated broth was streaked to mannitol salt agar and incubated overnight at 37 C. Colonies suspected of being *S. aureus* were picked for testing for coagulase production, using the tube method. Samples of stock and homogenized turkey were streaked directly onto mannitol salt plates and examined as indicated for the swab samples.

[4]All laboratory work except the samples that were put into cooked meat dextrose broth were conducted by the Bacteriology Laboratory, Division of Physical Health, Georgia Department of Human Resources, Atlanta, Ga.

RESULTS AND DISCUSSION

Several temperature recordings were made at various stages of processing turkeys, turkey meat, stock, gravy, and dressing. Temperatures were similar under any one set of circumstances, so only one example was chosen to illustrate results. Discussion is based on the growth and survival of common foodborne pathogens that frequently contaminate turkeys *(5, 6)*. Special reference to *C. perfringens* is made because of the selective advantage it has over other pathogens in turkeys prepared in school food service operations.

Thawing

When frozen 20-lb. turkeys were put in a forced air, reach-in refrigerator, they were still frozen (27 F) after 40 h (Fig. 1). In contrast, when 20-lb. turkeys were thawed at room temperature, internal temperatures reached 32 F in about 9 h and reached 50 F in about 18 h; surface temperatures reached 50 F in 5 h and were 62 F at the end of the holding period (Fig. 1 and 2). When turkeys were thawed in double-layer, kraft paper bags, internal temperatures reached 32 F in about 16 h, and at the end of the holding period (22 h) the internal temperatures was 40 F and the surface temperature 55 F. The relationship of internal temperatures to surface temperatures during thawing is illustrated in Fig. 2.

Thawing turkeys in a refrigerator is satisfactory, but it takes several days *(26)*. Thawing plastic-wrapped turkeys at room temperature in paper bags can be satisfactory *(17)*, but, as a rule of thumb, the time for thawing should not exceed 1 to 1-1/4 h per pound. Thawing plastic-wrapped turkeys not enclosed in paper bags overnight at room temperature is not a satisfactory method because psychrotrophic bacteria could multiply during the holding period *(26)*. However, because of the relatively low temperatures of the thawing turkeys and the competitive advantage of psychrotrophic bacteria, it is doubtful that, under the conditions of the experiment, *C. perfringens, S. aureus,* or *Salmonella* could multiply to a great extent, if at all, in the turkeys.

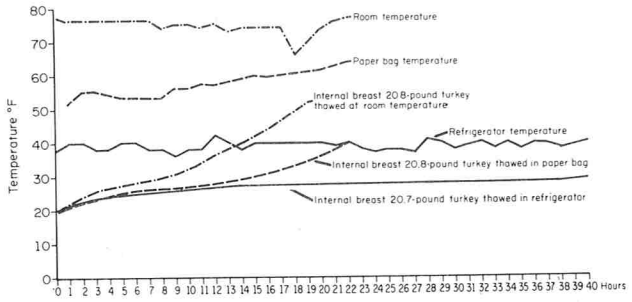

Figure 1. Comparison of internal breast temperatures of plastic wrapped 20-pound turkeys during thawing at room temperature, in a double-layer Kraft paper bag at room temperature, and in a forced-air reach-in refrigerator.

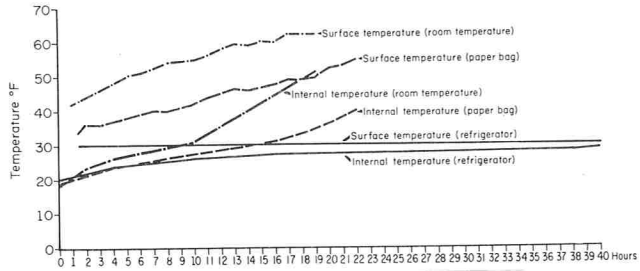

Figure 2. Relationship of surface temperatures to internal temperatures of plastic wrapped 20-lb. turkeys during thawing at room temperature, in a double-layer Kraft paper bag at room temperature, and in a forced-air reach-in refrigerator.

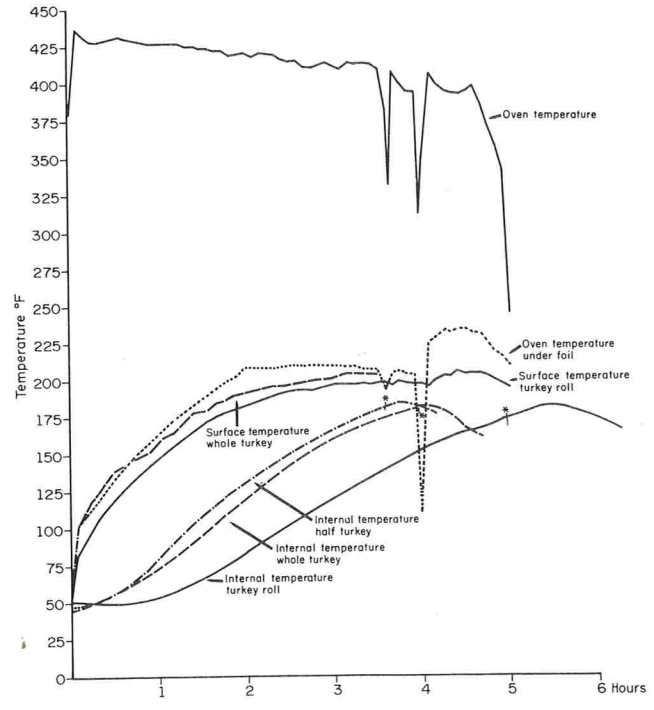

Figure 3. Comparison of baking whole turkey (20 lb., 3 oz.), half turkey (11 lb., 2 oz.), and turkey roll (13 lb., 7 oz.) in pans with foil tent in the same oven. *End of cooking.

Raw turkeys

A greater number of raw turkeys were contaminated with *C. perfringens* than with *S. aureus* or *Salmonella* (Table 1). One culture of *S. aureus* that was isolated from raw turkeys produced enterotoxin D. *C. perfringens* was also found in a sink in which raw turkeys had been washed. Hands and food-contact surfaces of equipment can become contaminated when they touch raw turkeys. Cooked meat, stock, gravy, dressing, or other foods that subsequently contact these surfaces can become contaminated.

Cooking

When whole turkeys, half turkeys, and turkey rolls were roasted in conventional gas ovens, all at the same temperature, turkey rolls took longer to cook because of their shape and bulk (Fig. 3). A 13-lb., 7-oz. turkey roll that was made from a 20-lb turkey, for instance, took about 1-1/2 h longer to reach 175 F than for a whole, 20-lb., 3-oz. turkey to reach the same temperature. Cutting turkeys in half decreased the cooking time by only 15 to 20 min. Comparable results from roasting half and whole turkeys and turkey rolls are reported in articles by Alexander et al. *(1)*, Iacono et al. *(16)*, Esselen et al. *(12)*, Goertz and Stacy *(13)*, Augustine et al. *(2)*, Hoke et al. *(15)*, and Wilkerson et al. *(37)*.

Surface temperatures rose rapidly in comparison to internal temperatures. All surfaces that were checked reached 165 F in less than 1-1/2-h baking time (Fig. 3); this temperature was usually reached in less than 30 min. In one situation, the area just under the skin of a turkey roll was tested; this area reached 165 F in 2-1/4 h whereas it took 5-1/2 h for the center of the roll to reach the same temperature (data not illustrated).

In forced-convection electric ovens, unwrapped turkeys cooked more rapidly than did foil-shielded turkeys roasted in conventional ovens when temperatures of about 325 F were used (Fig. 4). When low temperatures (200 to 225 F) were used, even in the forced-convection ovens, long periods of roasting were required—too long to be practicable in a school food service operation. In the trial illustrated in Fig. 4, for instance, baking was terminated before an internal temperature of 165 F was reached. Turkeys were cooked rather rapidly in steam kettles. The internal temperatures reached 165 F in approximately 2-1/2 h (Fig. 5).

When turkeys are cooked in ovens at temperatures of 325 F or higher, in steam kettles, in steamers, and in pots of boiling water long enough so that temperatures of 165 F are reached in the thickest part

TABLE 1. FOODBORNE PATHOGENS ISOLATED FROM RAW AND COOKED TURKEYS AND FROM SCHOOL LUNCH ENVIRONMENTS

Sample	*Clostridium perfringens*	*Staphylococcus aureus*	*Salmonella*
Raw products			
Raw turkeys	10/24[a]	5/24[b]	4/24
Thawed drippings	0/1	0/1	0/1
Spices	0/2	—[c]	—
Equipment			
Pots and pans (before use)	2/4	—	—
Pans (after use)	0/1	—	—
Glass jars (for stock)	1/3	0/2	0/2
Cutting boards (before use)	1/2	0/1	0/1
Cutting boards (after use)	2/3	0/1	1/1
Wooden paddle	0/1	—	—
Knives	0/2	0/1	0/1
Saw	0/1	—	—
Sink (before use)	0/1	0/1	0/1
Sink (after washing turkeys)	1/3	0/2	0/2
Cooked products			
Cooked turkeys	3/13	0/4	0/4
Chopped giblets	0/1	0/1	0/1
Stock	2/4	—	—
Dressing	0/1	—	—
Turkey and gravy	0/1	—	—
Totals	22/68	5/39	5/39

[a]Number of positive samples/total number of samples
[b]One isolate produced enterotoxin D
[c]—No laboratory tests performed

of the breast, vegetative bacteria within the meat should be killed, but bacterial spores could survive. Vegetative bacteria on outside skin surfaces would usually be destroyed within an hour. This would not be true, however, of bacteria on surfaces of the internal cavity of the turkeys.

Holding and chilling

There is little or no opportunity for foodborne pathogens to grow on or in turkeys for 1 to 2 h after turkeys have been cooked to high internal temperatures—even when held at room temperature or in unheated ovens (Fig. 3, 4, 5, 6). After turkeys are taken out of ovens or kettles and put at room temperature, an initial post-oven temperature rise occurs. Increases of 2 to 8 F were observed (Fig. 3, 4, 5).

If turkeys are held at room temperature or in unheated ovens for long periods, however, hazardous situations develop and have resulted in foodborne outbreaks. Such a situation was demonstrated when a half turkey remained in an unheated oven overnight (Fig. 6). During this storage, the surface and interior remained near the optimum growth temperature for foodborne pathogens for over 11 h. This amount of time at such temperatures allows spores of heat resistant *C. perfringens* that survive cooking to germinate and allows the resulting vegetative cells to multiply. Some spores of *C. perfringens* have survived boiling for over 4 h *(3, 14)*. If recontamination occurs by handling or during transferring pans or testing for doneness, these organisms would also have an opportunity to multiply to enormous numbers. The practice of cooking turkeys late in the day or late in the evening and allowing the turkeys to stay in unheated ovens overnight is a hazardous one and should be discontinued.

When whole cooked turkeys were put in refrigerators, the turkeys' internal temperature remained for 10 h or longer within a range that would allow foodborne pathogens to multiply (Fig. 7). These results agree with those of Lewis et al. *(19)* who observed that chickens which were cut up immediately

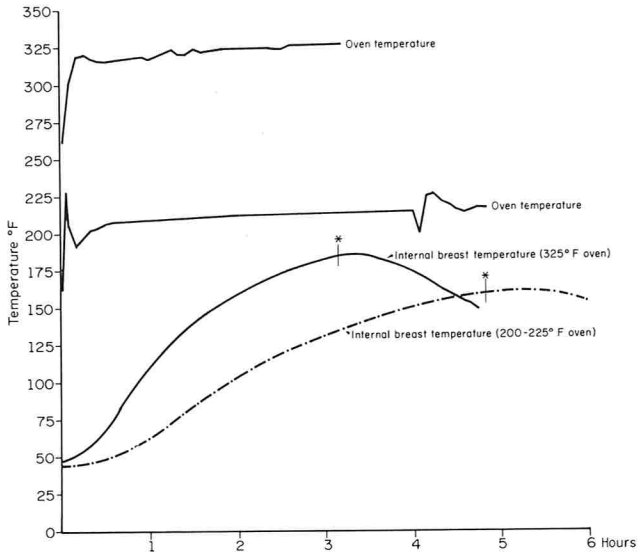

Figure 4. Baking 20-lb. turkeys in forced-convection electric oven at different temperatures. *End of cooking.

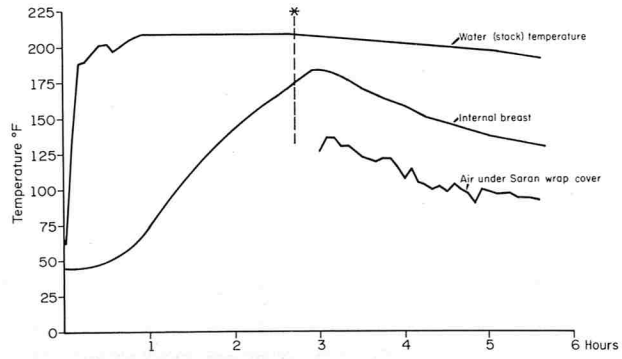

Figure 5. Cooking turkeys in steam kettle and cooling turkeys in pans under a Saran wrap at room temperature. *End of cooking. Turkeys out of steam kettle to pans on table; stock held in kettle with heat turned off.

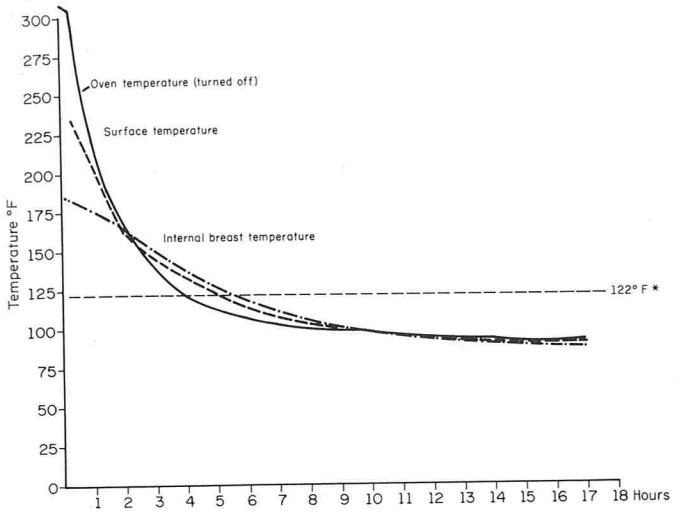

Figure 6. Overnight storage of a half turkey (9 lb., 15 oz.) in an oven that had been turned off. *Temperature at which *Clostridium perfringens* commences to multiply.

after cooking cooled faster than did whole cooked chickens when both were simultaneously refrigerated.

Turkey rolls are a greater potential foodborne disease hazard than are whole turkeys. While deboning turkeys and preparing rolls, contamination from the turkey surface as well as contamination introduced by workers and equipment, readily gets incorporated into the internal parts of rolls. Because of the bulk of the rolls and, consequently the greater the time required for cooking, chances of undercooking a roll are greater than are chances of undercooking a whole turkey. Slow heat transfer occurred in one-half a turkey roll that was stored in a walk-in refrigerator. The 4.6-lb. half roll remained within the growth range (60 to 122 F) for *C. perfringens* for 6 h (Fig. 8). In another trial, a 5.25-lb. half roll was within the growth range for this organism for 7 h when stored in a reach-in refrigerator that was operated at about 50 F (data not illustrated). The rolls would remain in the growth range (44 to 114 F) for staphylococci and salmonellae for an even longer time.

Because of the slow rate of heat transfer in cooked whole and half turkeys and in cooked turkey rolls during refrigerated storage, methods were devised to rapidly chill them. One method of chilling that resulted in rapid cooling was to put cut-up pieces of cooked turkey meat (8.5 lb.) into a pan, the bottom of which was in direct contact with ice placed in another pan (Fig. 9). The temperature of the meat was reduced to about 70 F in an hour. Rapid chilling of the meat in this way, followed by storage in a walk-in refrigerator, indicated that the center temperatures of 8-1/2 lb. of meat could be reduced in 3 h to below the minimum temperature that permits *C. perfringens* to grow; the temperature could also be reduced below 50 F in a little over 5 h, which for practical purposes would prevent the growth of *S. aureus* and *Salmonella* (Fig. 10). Other methods used to rapidly cool the same amount of meat were: putting meat into shallow pans and then placing in a refrigerator or freezer or putting the meat into double plastic bags and immersing in a pan of ice (Fig. 9). When 8.5 lb. of cut-up meat was put directly into a refrigerator, 60 F was attained in 3-2/3 h and 50 F in 7 h (Fig. 10). Advantages of chilling foods in shallow pans instead of in stockpots and bowls have been shown by Lewis et al. *(19)*, Weiser et al. *(36)*, and McDivitt and Hammer *(24)*.

The advantages of chilling turkey meat in an ice bath is illustrated in Fig. 8. Half a turkey roll was put into a double plastic bag which was put in a pot, covered with ice, and then refrigerated; the other half was put directly into a refrigerator (45 F). The half roll in the ice cooled from 160 to 60 F in 2-1/2 h, but the half put directly into the refrigerator required 7 h for the same drop in temperature.

Refrigeration of turkey stock in stockpots is, at best, hazardous because large volumes cool extremely slowly *(4, 21)*. For instance, 12.4 gal of stock in a 14-gal stockpot remained within the growth range for *C. perfringens* from the time the stock cooled to the maximum growth temperature (122 F) until the time it was used—about 13 h later (Fig. 11). Even in much smaller volumes (2.4 gal in a 5-gal stockpot), the stock temperature remained in the growth range for *C. perfringens* for 9-1/3 h, and in the growth range for *S. aureus* and *Salmonella* for even longer (Fig. 11). Stock stored in jars of only 1 gal capacity took over 7 h to cool to 60 F *(10)*. Turkey stock is an ideal medium for *C. perfringens* and a good medium for *S. aureus* and *Salmonella,* and, after it is cooked and stored in a large stockpot, anaerobic conditions which permit the growth of all of these organisms exist.

Stock should be rapidly cooled before it is refrigerated to prevent bacterial growth that can occur

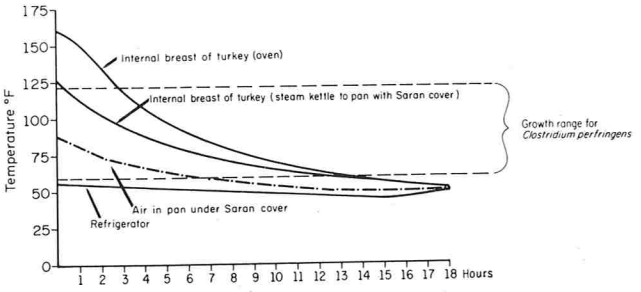

Figure 7. Chilling of whole 20-lb. cooked turkeys in a walk-in refrigerator; one directly from oven, the other after cooling at room temperature.

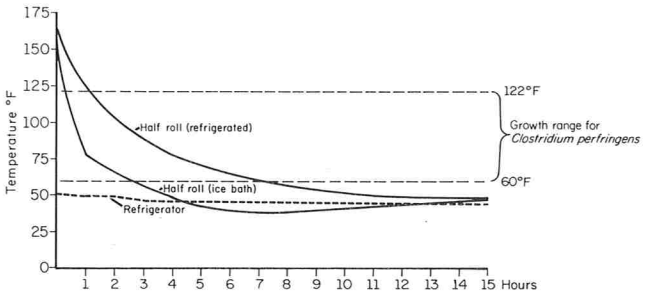

Figure 8. Chilling of two halves (4.6 lb. each) of a turkey roll in a walk-in refrigerator; one half in a refrigerator, the other in a plastic bag which was put in an ice bath and then into a refrigerator.

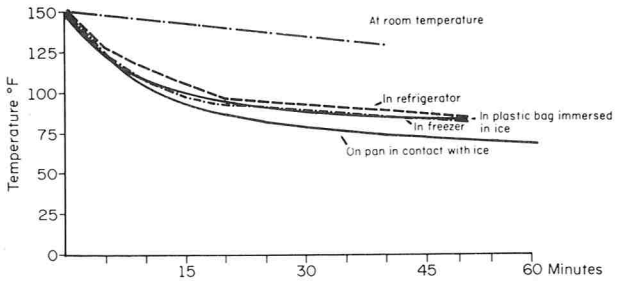

Figure 9. Rapidly chilling pieces of cut-up, cooked turkey put directly in a refrigerator, in a freezer, in a plastic bag which was immersed in ice, and in a pan which was put in contact with ice.

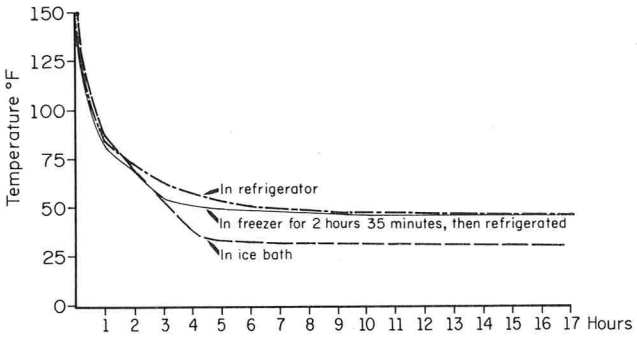

Figure 10. Chilling and storage of turkey meat in a walk-in refrigerator, in freezer and then refrigerator, and in an ice bath.

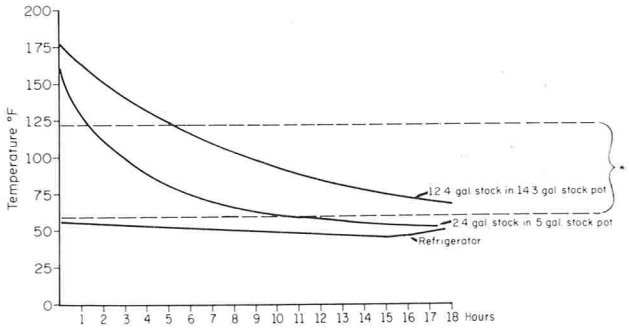

Figure 11. Chilling of turkey stock in 5-gal and 14-gal stockpots in a walk-in refrigerator. *Growth range for *Clostridium perfringens.*

during refrigerated storage. Three methods of rapidly cooling stock were used to compare temperatures reached with those reached by cooling at room and refrigerator temperatures (Fig. 12). For each experiment, 2.4 gal of turkey stock at an initial temperature of 160 F were stored in a 5-gal stockpot. Two methods, placing stockpots containing stock in a pan filled with ice and into a running water bath, rapidly cooled the stock. In the ice bath, for instance, the temperature dropped from 160 to 60 F in 42 min (Fig. 12). Another method, mixing stock at low speeds for an hour, also aided in reducing temperatures faster than storage at refrigerator or at room temperatures. Methods of rapidly chilling liquid foods are discussed by several investigators *(20, 22, 23-25, 27-30).*

Cooked products

C. perfringens was isolated from both cooked turkey meat and stock. Their presence can be accounted for by either survival of heat-resistant spores or by post-cooking contamination. Staphylococci and salmonellae were not found in samples of cooked turkey, but *Salmonella heidelberg,* the same serotype isolated from three of four swab samples from raw turkeys, was isolated from a cutting board. *C. perfringens* was found on cutting boards, before and after use, and in pots, pans, and jars that were subsequently used as containers for cooked turkey meat and stock (Table 1). The opportunity for contamination of cooked turkeys with foodborne pathogens is indicated by these findings.

If equipment that contacts raw turkeys is inadequately washed and disinfected, salmonellae, *C. perfringens,* or *S. aureus* may be present; if equipment is not stored so as to be protected from contamination, the ubiquitous *C. perfringens* may be present. Cooked turkey meat or stock can become contaminated upon contact with equipment or when put into containers which are so contaminated. Because cooking kills competitive vegetative bacteria but allows heat-resistant spores to survive and aids in establishing anaerobic conditions, *C. perfringens* can multiply to large numbers if turkey or stock are improperly chilled and stored.

Reheating stock and turkey meat

Because of the slow rate at which turkey stock cools, it is essential that it be heated to boiling or at least 165 F on the day it is used. Such heating will kill any vegetative bacterial cells that may have multiplied during storage. Figure 13 illustrates temperatures reached in stock and in gravy which was prepared from stock as they were cooked on a range, and Fig. 14 shows that boiling temperatures were

reached when stock and gravy were cooked in a steam kettle. In some school food service operations, however, it was not a routine practice to heat stock to such a high temperature *(10)*.

Before turkey meat is served, it should also be heated to a temperature that will kill vegetative bacteria. This cannot be accomplished by pouring hot gravy over cold meat or by heating cold meat on a serving table. The meat or mixture of meat and gravy needs to be heated to at least 165 F. Because of time pressures that occur in a school kitchen when turkey is served, chilled meat is frequently not heated enough to kill organisms that multiplied during storage.

When pans of meat and gravy were cooked on a range and in ovens for a sufficient period of time, the mixture reached temperatures that would kill vegetative bacterial cells (Fig. 13). Heating in ovens, however, is not always done for long enough time to be bacteriologically safe *(10)*. When pans of meat and gravy were heated on a range, it was difficult to ensure adequate heat treatment of meat in the upper parts of the pan until lids were used or until the pans were transferred to ovens.

When cold meat was put into a steam kettle of boiling gravy, the meat reached 165 F in 15 to 20 min. Adequate terminal heat treatment was also achieved by heating meat in pressure-type steamers. The temperatures reached are illustrated in Fig. 14.

Baking dressing

Dressing that was baked in 2-inch-deep pans reached internal temperatures above 200 F at the end of baking. This process would be lethal to vegetative bacterial cells *(11, 12, 15, 31, 35)*.

SUMMARY

To ensure safety of turkey products in school food service operations, thawed turkeys should be cooked until internal temperatures are at least 165 F. Cooked turkeys should be deboned and cut into small pieces as soon as they cool to a point where they can be handled. Methods should be used to rapidly chill both turkey meat and stock, and the prechilled products should be stored until used in a refrigerator which has a temperature below 45 F. Rapid cooling of cooked products is the most important step in preventing outbreaks of foodborne illness from turkeys, but stock should be boiled and turkey meat reheated to at least 165 F before serving to nullify inadequacy in cooling practices.

RECOMMENDATIONS

Based on this investigation, the following recom-

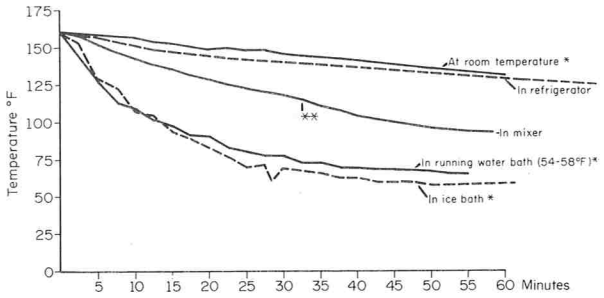

Figure 12. Chilling turkey stock (2.4 gal) in a 5-gal stock pot in an ice bath, water bath, mixer, refrigerator, and at room temperature. *Stirred every 5 min. **Changed speed from 1 to 2.

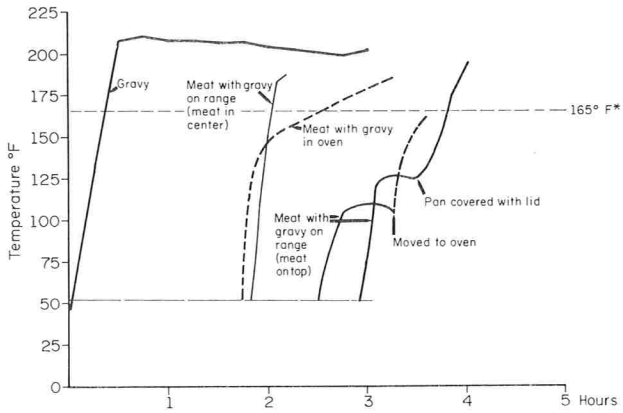

Figure 13. Cooking giblet gravy and reheating turkey and gravy in oven (325 F) and in open and covered pans on a range. *Temperature at which vegetative should be killed.

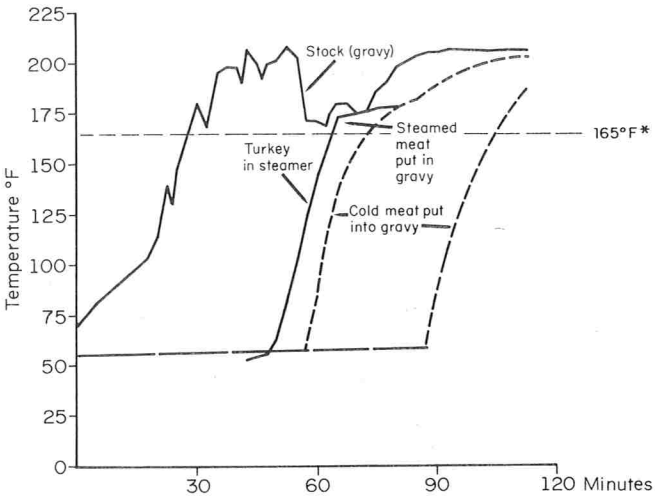

Figure 14. Reheating stock and gravy in a steam kettle, heating turkey meat in gravy in a steam kettle, and heating cold turkey meat in a steamer. *Temperature at which vegetative bacteria should be killed.

mendations are made for preparing, cooking, cooling, and reheating turkeys in a school kitchen:

Thawing

1. Always thaw frozen turkeys completely before cooking them. Too much time is required to adequately cook large, frozen turkeys to be practicable in school food service operations.
2. Thaw plastic-wrapped turkeys in refrigerators whenever possible. The refrigerator temperature should be 45 F or below. (For 20-lb. turkeys, 3 days or more are required.)
3. If turkeys must be thawed within 24 h or if refrigerator space is not available, enclose wrapped, frozen turkeys in double kraft paper bags, tape the bags shut, and thaw at room temperature. When thawing in bags, do not allow them to remain at room temperature more than 1 h per pound if one, double-layer bag is used or 1-1/4 h per pound if two, double-layer bags are used.

Cooking

1. Cook turkeys to internal temperatures of at least 165 F. (180 to 185 F and 170 to 180 F are recommended for thigh and breast internal temperatures, respectively.)
2. Test turkeys for doneness by inserting a thermometer into the center of turkey rolls.
3. Bake dressing separately until it reaches an internal temperature of at least 165 F.
4. When baking turkeys in ovens, set thermostat at 325 F or higher. Baking turkeys at temperatures of 225 F or lower requires too much time for the turkeys to reach an internal temperature of 165 F to be practicable in a school food service operation.

Holding and cooling

1. Never allow cooked turkeys to stay in unheated ovens (such as for overnight holding).
2. Never refrigerate cooked whole turkeys for overnight storage without first reducing their bulk.
3. Wear disposable plastic gloves when deboning cooked turkeys.
4. As soon as the turkeys cool to a temperature at which they can be handled, debone them and slice or cut meat into small pieces.
5. Put pieces of cut-up, cooked turkeys directly into cold pans (pans setting in pans of ice).
6. Do not pile turkey meat more than 3 inches high in pans.
7. Never store turkey meat and stock in the same containers.
8. Never refrigerate large batches of turkey stock in large stockpots.
9. Rapidly chill stock by immersing containers, such as 2-1/2 gal in a 5-gal stockpot, in an ice bath or a water bath, or by mixing with a vertical mixer before storing in a refrigerator.

Reheating

1. Boil stock on the day it is to be used either before or during gravy preparation.
2. Reheat turkey meat to 165 F in steamers, in boiling gravy, in covered pans on a range, or in open pans in ovens. (This last procedure, heating meat with gravy in ovens, requires considerable time.)

Cleaning and personal hygiene

1. Thoroughly wash and disinfect all equipment that touches raw turkeys before it is used for cooked meat or stock.
2. Wash hands after handling raw turkeys and before handling cooked turkey or other foods.

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EFFECT OF LOW DOSE GAMMA IRRADIATION ON GROWTH AND AFLATOXIN PRODUCTION BY *ASPERGILLUS PARASITICUS*[1]

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Abstract

Spores and growing vegetative mycelia of *Aspergillus parasiticus* strains NRRL 2999 and NRRL 3000 were irradiated at 100 and 200 Krad, and the effects on growth and aflatoxin production in yeast-extract sucrose (YES) broth were measured. Irradiation of growing mycelia reduced subsequent growth in YES broth by a greater amount than irradiation of spores. Irradiation of spores at 100 Krad resulted in more B_1 and G_1 production by strain NRRL 2999 than the non-irradiated control, however, strain NRRL 3000 produced less aflatoxins B_1 and G_1 after irradiation at 100 Krad than its non-irradiated control. Spores of both strains irradiated at 200 Krad produced less aflatoxins B_1 and G_1 than non-irradiated controls. Irradiation of growing vegetative mycelia of both strains at 100 and 200 Krad resulted in a definite decline in both aflatoxins B_1 and G_1 in subsequent cultures at each irradiation level. Apparent stimulation of production of both B_1 and G_1 occurred after irradiation of spores of strain NRRL 2999 at 100 Krad. However, the variation of the values as determined by the standard deviation was such that one would conclude that no differences existed among means. The apparent stimulation was slight and of much less magnitude than that which has been reported by other investigators using *A. flavus*. No stimulation of toxin production was observed with the other strain when grown from irradiated spores or with either strain when vegetative mycelia were irradiated.

Low level ionizing irradiation, amounting to pasteurizing doses to extend shelf-life, have been studied extensively as a means of preventing bacterial deterioration of food products *(5)*. However, few reports of the effects of low level gamma irradiation on toxic molds are available. Mohyuddin et al. *(9)* studied inactivation of conidiophores and mycelia of *Aspergillus flavus* by gamma irradiation, but did not measure the effects on aflatoxin production. Jemmali and Guilbot *(6, 7, 8)* reported that gamma irradiation does below 200 Krad may induce or increase aflatoxin production in *A. flavus*, when grown on Czapek's broth fortified with yeast extract. They found the effects to be maximum at 100 and 150 Krad after 7 days incubation at 25 C. On the other hand, Applegate and Chipley *(1)* observed increased aflatoxin B_1 production by *A. flavus* at 150, 200, and 300 Krad of irradiation when the organism was grown on a cracked wheat substrate. They also found that only the 150 Krad dose stimulated aflatoxin B_1 production by *A. flavus* in a synthetic medium. Production of aflatoxin G_1 was affected in a similar manner *(2)*. Growth of toxic and non-toxic strains of the fungus in wheat and synthetic media was greatly reduced by exposure of the organisms to 300 Krad. Applegate and Chipley *(1, 2)* did not observe induction of the ability to produce either aflatoxin B_1 or G_1 in nontoxic strains by irradiation. Schindler and Noble *(10)* observed increased B_1 production by an *A. flavus* strain after exposure to 20, 100, 250, and 500 kr. In one instance the maximum was observed at 100 kr and in another at 500 kr. At 500 kr the increase was more than 50-fold over the non-irradiated control. They also observed stimulation of G_1 production at the 100 and 500 kr levels. However, in another study Schindler et al. *(11)* observed no stimulation of aflatoxin production at 49-53 Krad of irradiation of both *A. flavus and Aspergillus parasiticus*. Because of the difficulty of converting kiloroentgens (kr) to kilorads (Krad), it is not possible to directly compare these results with those of others. However, it is evident that in the studies of Schindler and Noble *(10)* the greatest stimulation of aflatoxin production occurred at a higher level of irradiation than observed by Jemmali and Guilbot *(6)*.

While stimulation of aflatoxin production by *A. flavus* after irradiation has been observed by several workers, there are differing reports as to the exact level of irradiation that will cause the phenomenon. Most of the work reported to date has involved the effects of irradiation on *A. flavus* rather than *A. parasiticus*, except in one instance where no stimulation of toxin production by *A. parasiticus* was observed at very low levels of irradiation *(11)*. Toxic strains of *A. parasiticus* are generally more potent aflatoxin producers than are toxic strains of *A. flavus*. Also, *A. flavus* strains frequently produce only the B group of aflatoxins, and not the G group. Schindler *(12)* has found that different strains of *A. flavus* vary greatly in their response to irradiation. It is not known whether *A. parasiticus* would react in the same manner to irradiation as *A. flavus*. While *A. flavus* and

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A. parasiticus are generally recognized as morphologically distinct, few physiological differences are known *(15)*. Yet, differences in patterns of aflatoxin production between the two organisms clearly exist *(4, 15)*.

Additional studies would be helpful to adequately assess the effects of low level ionizing irradiation on different aflatoxin producing mold strains, particularly strains of *A. parasiticus*. This work was initiated to study the effects of low level gamma irradiation on growth and aflatoxin production by known aflatoxinogenic strains of *A. parasiticus*.

MATERIALS AND METHODS

Media for growth and toxin production

Yeast-extract sucrose (YES) broth *(3)* was used to support growth and aflatoxin production by the organisms in still cultures before and after irradiation treatment. Aliquots of 50 ml of the broth contained in 250 ml erlenmeyer flasks were employed.

Organisms, inoculum, and treatments.

A. parasiticus strains NRRL 2999 and NRRL 3000 were obtained from the culture collection of the Northern Regional Research Laboratory, A.R.S., U.S.D.A., Peoria, Illinois, for this study.

Cultures of the toxic molds were grown on potato dextrose agar (Difco) slants for 10 days at 25 C until well sporulated. The spores were washed from the slants with a sterile 0.01% solution of Tween 80 and aseptically filtered through sterile cheese cloth to remove mycelial debris. Spores in the filtered suspensions were counted using a Petroff-Hausser counting chamber. A portion of each spore suspension was diluted with sterile phosphate buffer to obtain a spore concentration of 10^7 conidia/ml. Aliquots of 10 ml of the diluted spore suspensions were irradiated at 0, 100, and 200 Krad at ambient (ca 25 C) temperature using a Cobalt-60 source similar to the one described by Teeny and Miyauchi *(14)*. Dosimetry for irradiating the inocula was established using the Fricke Dosimeter, ASTM D1671-63. Flasks containing sterile YES broth were inoculated with 0.1 ml of the irradiated or non-irradiated spore suspensions, resulting in approximately 10^6 spores per flask culture.

The effects of irradiation on growing vegetative mycelia and subsequent growth and toxin production in broth cultures were also studied. Slant cultures of the same two strains were grown on potato dextrose agar at 25 C until mycelial growth had just become evident (24-48 h), but before sporulation had begun. Slant cultures were irradiated at the same levels and in the same manner as were the spore suspensions. Several transfer loops of irradiated or non-irradiated mycelia were used to inoculate flasks of YES broth.

Determination of growth and toxin production after irradiation

All cultures were incubated as still cultures at 25 C for 7 days, and then given a brief heat treatment (121 C for 30 sec) to kill spores and vegetative mycelia. The mold mats were separated from the broth by gravity flow filtration through Whatman #4 filter paper and washed with 20 ml distilled water, dried at 130 C for 2 h and weighed. Mold growth was expressed as milligrams of mycelial dry weight. The pH of the filtered broth was determined.

Aflatoxins were extracted from the broth by liquid-liquid extraction using two 50 ml portions of chloroform. The extracts of each culture were filtered, pooled, and dried over 5-10 g of anhydrous sodium sulfate. The pooled extracts were either diluted or concentrated as needed in preparation for aflatoxin analyses. The aflatoxin concentrations in the extracts were estimated by visual comparison of the fluorescence of the samples to known standards on exposure to long wave U.V. light using thin-layer chromatography (TLC). The TLC plates were 20 × 20 cm, and were coated with a 0.25-mm thick layer of Silica Gel G-HR (Brinkmann Instruments, Inc.). The TLC plates were developed in toulene/ethyl acetate/formic acid (60/30/10) according to the method of Scott et al. *(13)*. Standard aflatoxins were obtained from the Southern Utilization Research and Development Division, U.S.D.A., New Orleans, LA.

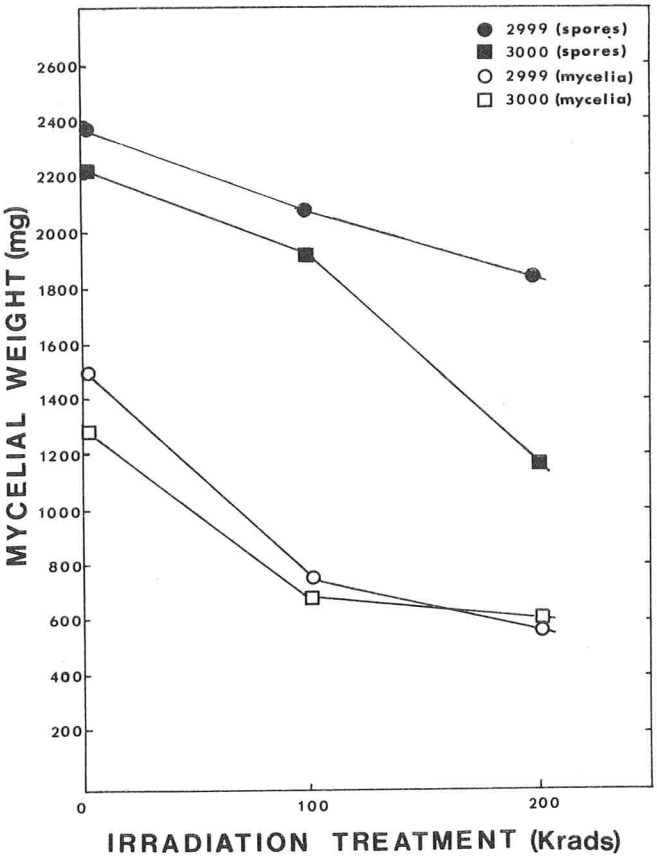

Figure 1. Effect of gamma irradiation of spores and vegetative mycelia of *Aspergillus parasiticus* NRRL 2999 and 3000, on subsequent amount of growth in yeast-extract sucrose broth after 7 days incubation at 25 C.

RESULTS

Growth

Low level gamma irradiation of both spores and growing vegetative mycelia partially inhibited growth of the two strains studied (Fig. 1). At the 200 Krad treatment, growth of strains NRRL 2999 from irradiated spores was reduced by 23% from the non-irradiated control. Irradiation of growing mycelia of the same strain at 200 Krad reduced growth by 62%. The growth of strain NRRL 3000 from spores irradiated at 200 Krad was reduced by 48% and from irradiated growing mycelia by 54% from the non-

TABLE 1. PRODUCTION OF AFLATOXIN B_1 BY *Aspergillus parasiticus* NRRL 2999 AND NRRL 3000 ON YEAST-EXTRACT SUCROSE BROTH IN 7 DAYS OF INCUBATION AT 25 C WHEN GROWN FROM IRRADIATED SPORES OR MYCELIA

Irradiation level (Krad)	NRRL 2999		NRRL 3000	
	Spores	Mycelia	Spores	Mycelia
	——(Aflatoxin B_1, μg/ml broth)——			
0 (Control)	136	88	60	17
100	150	13	43	5
200	120	4	17	<1

TABLE 2. PRODUCTION OF AFLATOXIN G_1 BY *Aspergillus parasiticus* NRRL 2999 AND NRRL 3000 ON YEAST-EXTRACT SUCROSE BROTH IN 7 DAYS OF INCUBATION AT 25 C WHEN GROWN FROM IRRADIATED SPORES OR MYCELIA

Irradiation level (Krad)	NRRL 2999		NRRL 3000	
	Spores	Mycelia	Spores	Mycelia
	——(Aflatoxin G_1, μg/ml broth)——			
0 (Control)	689	411	932	191
100	727	60	824	34
200	351	17	146	<1

TABLE 3. EFFECT OF LOW LEVEL GAMMA IRRADIATION OF SPORES AND MYCELIA ON THE SUBSEQUENT PRODUCTION OF AFLATOXIN B_1 PER UNIT WEIGHT OF DRY MOLD MAT BY *Aspergillus parasiticus* NRRL 2999 AND *NRRL* 3000 IN YEAST-EXTRACT SUCROSE BROTH AFTER 7 DAYS AT 25 C

Treatment (Krads)	NRRL 2999		NRRL 3000	
	Spores	Mycelia	Spores	Mycelia
	——(Aflatoxin B_1, μg/mg dry mycelium)——			
0 (Control)	2.9	2.6	1.3	0.6
100	3.6	0.7	1.1	0.3
200	3.1	0.3	0.7	<0.1

TABLE 4. EFFECT OF LOW LEVEL GAMMA IRRADIATION OF SPORES AND VEGETATIVE MYCELIA ON THE SUBSEQUENT PRODUCTION OF AFLATOXIN G_1 PER UNIT WEIGHT OF DRY MOLD MAT BY *Aspergillus parasiticus* NRRL 2999 AND NRRL 3000 IN YEAST-EXTRACT SUCROSE BROTH AFTER 7 DAYS AT 25 C

Treatment (Krads)	NRRL 2999		NRRL 3000	
	Spores	Mycelia	Spores	Mycelia
	——(Aflatoxin G_1, μg/mg dry mycelium)——			
0 (Control)	14.5	12.1	19.6	6.1
100	17.4	3.2	17.5	1.9
200	9.4	1.3	0.5	<0.1

irradiated controls. The final pH of the broth media in which the control cultures were grown was 5.3 while the pH of the cultures irradiated at 100 Krads were 6.0 and 6.2 for NRRL 2999 and NRRL 3000 respectively, and 6.2 and 6.5, respectively, for the cultures irradiated at 200 Krad. Growth of organisms in broth media is associated with a decline in pH as growth proceeds. The higher pH of the irradiated cultures reflects less growth which was borne out by the lower mycelial weights obtained.

Aflatoxin production

Aflatoxin B_1 production by cultures from spores of strain NRRL 2999 irradiated at 100 Krad was slightly more than the control (Table 1). At the 200 Krad treatment of spores, this strain produced less aflatoxin B_1 than the control. When growing vegetative mycelia of the same organism were irradiated at the 100 and 200 Krad levels, there was a definite decline in aflatoxin B_1 production at each level of irradiation (Table 1). Aflatoxin B_1 production by strain NRRL 3000 was decreased in cultures grown from spores irradiated at 100 Krad (Table 1). Irradiation of spores of strain 3000 at 200 Krad reduced B_1 production by more than one-half of the control. With growing vegetative mycelia, there was likewise a decline in aflatoxin B_1 production at each level of irradiation (Table 1).

More aflatoxin G_1 was detected in cultures of strain NRRL 2999 that were grown from spores irradiated at 100 Krad than control cultures (Table 2). However, irradiation of spores at 200 Krad resulted in almost 50% less G_1 production than the unirradiated control. Irradiation of growing vegetative mycelia of the same organism resulted in a decline in G_1 production at both the 100 and 200 Krad levels. Aflatoxin G_1 production by strain NRRL 3000 was decreased slightly after irradiation of spores at 100 Krad compared to the control (Table 2). At the 200 Krad treatment of spores, G_1 production was much less than the control cultures of the organism. Irradiation of strain NRRL 3000 mycelia caused a definite decline in aflatoxin G_1 production at both the 100 and 200 Krad treatments compared to the control (Table 2). At the 200 Krad treatment of growing mycelia very low levels of G_1 were produced.

To determine if there might have been stimulation of aflatoxin production by the irradiation treatments, the amount of toxins produced was converted to micrograms of toxins produced per milligram of dry mycelia. With strain NRRL 2999 spores, more aflatoxin B_1 was produced per milligram of dry mycelia at both the 100 and 200 Krad treatments than the control (Table 3). At 100 Krad the effect was greater than at 200 Krad. However, with irradiated growing mycelia of the same organism an opposite trend was noted, with a very definite reduction in amount of aflatoxin B_1 produced per milligram of dry mycelia. No stimulation of aflatoxin B_1 production by either irradiated spores or growing mycelia of strain NRRL 3000 was evident based on micrograms of toxin produced/milligram of dry mycelia (Table 3). There appeared to be a more or less steady decline in amount of B_1 production by this organism due to irradiation.

Aflatoxin G_1 production per unit weight of dry mycelia by cultures derived from irradiated spores of strain NRRL 2999 was greater than the control after irradiation at 100 Krad, but not after irradiation at 200 Krad (Table 4). Irradiation of growing mycelia

of NRRL 2999 caused a definite decline in the amount of G_1 produced per unit of dry mycelia at both the 100 and 200 Krad treatments compared to the control. Aflatoxin G_1 production per milligram of dry mycelia by irradiated spores of strain NRRL 3000 was less than the control cultures at both the 100 and 200 Krad treatments (Table 4). Irradiated growing mycelia of strain NRRL 3000 likewise showed a steady decline in the amount of G_1 produced per milligram of dry mycelia.

DISCUSSION

Growth

Strain NRRL 2999 spores seemed to be somewhat more resistant to irradiation at 200 Krad than those of strain NRRL 3000. Growth of strain NRRL 3000 from irradiated spores was reduced by a greater amount at this treatment than was growth of NRRL 2999 from irradiated spores. There did not appear to be much difference in the reaction of the two strains at 100 Krad since the decline in growth of both strains from that of controls appeared to be similar. The reduction of growth of *A. parasiticus* as a result of irradiation of spores supports and expands the observations with *A. flavus* of Applegate and Chipley *(1, 2)*. The retarding effect of irradiation on growth of cultures from irradiated growing mycelia was very evident at the 100 Krad level with further reductions at 200 Krad. Under the conditions of this study, the growing mycelia of both strains were more sensitive to irradiation in terms of reduced growth than were the spores.

Aflatoxin production

The only instance of possible stimulation of aflatoxin B_1 and G_1 production occurred with spores of strain NRRL 2999. The increased amounts were very small and were not of the same magnitudes as those observed by other workers with *A. flavus*. In addition the variation of these observations as determined by the standard deviation was such that it could be concluded that no actual differences existed between treatment means. The main effect was observed at 100 Krads, which is in agreement with the findings Jemmali and Guilbot *(6)*. However, the results of Schindler and Noble *(10)* and of Applegate and Chipley *(1, 2)* appear to be somewhat different since they observed increased aflatoxin production at higher levels of irradiation of *A. flavus*. However, Applegate and Chipley *(1, 2)* did not observe stimulation of aflatoxin production beyond 150 Krad when using a synthetic medium. Mohyuddin et al. *(9)* found only 0.15% survivors of *A. flavus* conidiophores irradiated at 125 Krad. Presumably higher irradiation levels would have resulted in almost complete inactivation of the particular strain of *A. flavus* they worked with, suggesting that aflatoxin production might also have been eliminated.

Strain NRRL 3000 did not exhibit any tendency toward stimulation of either B_1 or G_1 production at either the 100 or the 200 Krad treatment of spores. There seemed to be a more definite reduction in the amounts of both toxins produced by strain NRRL 3000 after irradiation at 200 Krad.

The irradiated growing mycelia of both strains did not exhibit any tendency toward increased production of either toxin as the result of irradiation treatment. The effect with growing vegetative mycelia was rather one of progressive decline in total toxin production as well as production of toxins on a per unit weight of mycelia. This might be explained by the fact that the mycelia are multinucleate structures, which would require a greater number of hits to produce genetic changes.

These studies show that low level gamma irradiation reduced the amount of growth of *A. parasiticus* in YES broth in a 7-day incubation period. In addition, aflatoxin production was markedly reduced in this same period. Obviously some of the inoculum was destroyed by the irradiation treatment, which might have delayed the amount of growth and aflatoxin production obtained in a 7-day period changing the growth cycle. However, the growth itself appeared to be affected in that the cultures that developed after irradiation did not grow as profusely as did the controls. Whether the effect is due to changes in the metabolism of the organism or simply a delay in the growth cycle is not clear. However, it is clear that growth and aflatoxin production are reduced or prevented within a given time frame. The incubation time of 7 days is comparable to the incubation time used by other workers, and permits comparison of data from different sources.

Obviously differences in results exist between several workers. The differences are too great to be accounted for on the basis of methodology. These differences range from 50-fold increases in toxin production by *A. flavus* at approximately 500 Krad reported by Schindler and Noble *(10)* to essentially no increases in toxin production by *A. parasiticus* in our studies at 100 Krad with definite reductions in toxin production occurring after irradiation at 200 Krad. In addition, Jemmali and Guilbot *(6)* obtained no increase in aflatoxin production by *A. flavus* at 200 Krad and a definite decline in toxin production at 350 and 500 Krad. Applegate and Chipley *(1, 2)* on the other hand observed stimulation of aflatoxin B_1 and G_1 production by *A. flavus* at 200 and 300 Krads

using a cracked wheat substrate, but no stimulation beyond 150 Krad using a synthetic medium. It would seem that these apparent discrepancies might be explained on the basis of differences between species, differences between strains within species, and substrate effects.

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A STATISTICAL EVALUATION OF THE 3M BRAND AUTOMATIC COLONY COUNTER[1, 2]

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Abstract

The 3M Brand Automatic Colony Counter, Model 620, was evaluated and compared with three laboratory technicians on raw milk Standard Plate Count (SPC) cultures over the range of counts from 0-300 colonies per culture plate. Overall, the estimated total variance for the three technicians was 15.07, for the 3M unit 17.82. On plates averaging greater than 150 colonies the 3M unit and hand counts were about equal in variability. On lower counts the 3M device was somewhat more variable, although within what would appear to be acceptable limits. For the range of data studied, 99.4% of the variance (for the average of three counts by either method) could be accounted for by a similar average of observations using the other method. The 3M unit averaged 2.33 counts higher than technician counts.

Counting of bacterial culture plates is a slow, tedious process at best, involving a certain degree of imprecision. Advent of automatic scanning and counting devices now make it possible, if counting accuracy is acceptable, to do this task electronically and at significant savings in time and labor, especially in large centralized laboratories.

In *Standard Methods for the Examination of Dairy* Products *(1)*, it is suggested [referring to work of Courtney *(2)* and Donnelly et al. *(3)*] that laboratory workers should have sufficient counting proficiency to duplicate counts on the same plate to within 5%, and counts of other analysts to within 10%. One can assume, therefore, that 10% error is the maximum tolerable, though better precision is, of course, more desirable.

The study reported herein analyzes and compares counting variability of the 3M Automatic Colony Counter with three laboratory technicians within ranges over the broad range of counts generally considered acceptable for the SPC (30-300 colonies).

Materials and Methods

Standard Plate Count (SPC) culture plates of raw milk samples were segregated for this study from regular-run samples in the on-going operation of the Dairy Quality Control Institute, Inc. laboratory. Approximately 20 plates were randomly selected in the range of 0-49 colonies, and then approximately 20 for each 50-count increment above 49 up to and including 300 colonies. All plates were prepared and incubated according to procedures set forth in *Standard Methods for the Examination of Dairy Products (1)*.

Plates found to fall within an appropriate range of count were counted by the technician engaged in this routine on any given day of laboratory operation. The plate was then counted in triplicate on a 3M Brand, Model 620, Automatic Colony Counter (3M Company, New Business Ventures Division, St. Paul, Minnesota, 55101). Between counts on the 3M unit, plates were rotated approximately 60 degrees to assure a different orientation for each count. After an individual plate had been thus counted, it was set aside for hand counting by two additional technicians, each of whom made one count. Thus, each plate was counted three times by machine and three times by technicians. The same three technicians were used exclusively, and in all instances counting was completed within 2 h folowing the initial counts. All technicians used a hand tallying device for totaling counts.

In addition to the 120 plates counted in triplicate, six plates were selected for special study. Counts on these plates varied around the mid-range of the major incremental ranges selected for the study, that is between 50 and 99, 100 and 149, etc. Each of these plates were counted 20 times on the automatic counter, rotating the plate approximately 18 degrees between counts. Plates were then hand counted five times on five different occasions by a single technician. Counts were recorded and subjected to a special statistical analysis separate from that made on the other 120 plates. Variance estimates were determined. Also, the Behrens-Fisher test *(4)* was applied to each separate plate.

A few comments should be made concerning operational requirements of the 3M Brand, Model 620, Automatic Counter. Because the unit detects colonies by measuring changes in light transmitted through the agar medium, plates must be absolutely clean. No trademarks, pen or crayon marks can be present. Also, scratches will be "seen" and counted as colonies, causing inaccuracies. Plates should be periodically checked and those with scratches discarded. For this investigation, to minimize the possibility of scratches interfering with counts, plastic plates were used. Secondly, the agar must be fairly uniform in transparency and colonies distributed evenly over the surface. No special technique is required over and above methods outlined in *Standard Methods for the Examination of Dairy Products*. But the point is, sloppy laboratory procedures cannot be tolerated if best results are to be achieved. In this study, no speecial plating skills were applied. Plates were simply picked from among

[1]Scientific Journal Series Paper No. 8536, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55101.
[2]Reference to commercial products or trade names is made with the understanding that no discrimination is intended and no endorsement is intended.

TABLE 1. AVERAGE VARIANCES AND COEFFICIENT OF VARIATION VALUES FOR THREE TECHNICIANS AND THE 3M AUTOMATIC COLONY COUNTER OVER VARIOUS RANGES OF SPC COUNTS[1]

	Technicians				3M Automatic Counter			
Range of counts	No. of plates	Avg. count	Variance[2] ($\bar{s}^2$)	Coef. of variation[2]	No. of plates	Avg. count	Variance[2] ($\bar{s}^2$)	Coef. of variation[2]
0-49	28	29.9	1.42	0.0345	24	29.4	6.66	0.1144
50-99	22	73.8	4.30	0.0244	25	73.7	17.47	0.0516
100-149	20	126.6	9.65	0.0213	19	125.3	14.37	0.0273
150-199	19	175.9	34.50	0.0249	18	170.3	21.61	0.0237
200-249	20	223.2	19.70	0.0164	24	223.0	24.14	0.0189
250-300	19	275.5	20.37	0.0142	17	274.5	25.75	0.0109

[1]Counts were made in triplicate in all cases.
[2]Values for s^2 and coefficient of variation were computed separately for each plate. The table entry is the average of values for plates in the specified range.

the samples being routinely counted in the quality control laboratory.

Lastly, spreaders may overlap other colonies and interfere with the counting process. Such plates should either be counted manually, or the spreader and masked colonies counted separately and added to the tally observed by the automatic counter. To avoid this variable altogether, only non-spreader plates were analyzed in the work reported herein.

RESULTS AND DISCUSSION

After extracting variance due to plate differences, estimated total variance for manual counting was found to be 15.07. Variance of counts obtained with the 3M unit, after extracting the plate sum of squares, was 17.82, an indication that counts obtained electronically were somewhat more variable than technician counts.

In Table 1 may be seen variance and coefficient of variation values for both technicians and machine. With the exception of technician counts between 150-199 colonies, which appear to be out of line, machine and technician counting show about the same variability at counts above 150. Computation of the line of best fit by least squares criteria established the following relationships between the two counting methods. For the standard method

$$\hat{\sigma}^2_{SM} = 0.1257\ \hat{u}_{SM} - 2.9668, \text{ for the 3M unit } \sigma^2_{3M} = 0.634\ \hat{u}_{3M} + 8.5733.$$

Initially, the relationship between $\hat{m} = \frac{\hat{u}_{SM} + \hat{u}_{3M}}{2}$ and $\hat{d} = (\hat{u}_{SM} - \hat{u}_{3M})$ was computed, and was found to be: $\hat{d} = -4.0292 + 0.0118\ \hat{m}$. There was, on the average, a +2.33 colony count bias by the 3M method.

Over the range of data included in this investigation, 99.4% of the variance for a three count average by either method was accountable in a similar average of observations by the other method. Certainly, this is indicative of a high level of agreement between the two methods.

To further study the variability in counting methods six plates of widely different counts were selected. Each plate was counted 20 times by the automatic counter and five times by a single technician. Variance estimates were significantly larger than those predicted by the previous relationships at the 0.05 level for four plates using the 3M counter and one plate by the standard method. The Behrens-Fisher test *(4)* (applied separately to each plate) was used to determine whether or not the observed $\hat{d}$ was significantly different from −2.33. Results showed that four of the six plates were significantly different from this value. Clearly, other factors besides actual colony numbers affect the difference between counts by the two methods and, to a lesser extent, affect counting precision by either method.

The 3M unit shows greater variability at low counts, but only at counts below 50 does the variability appear to be of questionable magnitude. According to the estimated relationship σ 3M is 1/10 of $\hat{u}_{3M}$ when $\hat{u}_{3M} = 32.62$; it is 1/20 of $\hat{u}_{3M}$ when $\hat{u}_{3M} = 72.60$. In other words, the standard deviation is 10% of the mean (or 3.26 colonies) at a count of 32.62. When the count reaches 72.60, the standard deviation is 5% of the mean (or 3.63 colonies). In any event, the data do not provide for variability among laboratories. By the same token variability in the 3M counter does not include variability among machines. However, one might expect this latter source of variability to be rather small.

The variability of mean observed values is much larger than would be expected on the basis of variability estimated from deviations around the mean for each plate. This observation, along with the somewhat inconsistent results obtained when a large number of replicates was counted, strongly suggests that certain characteristics of the plates other than actual numbers of colonies influence the variability of counts by both methods. Actual precision, by

either procedure, is likely similarly affected by extraneous characteristics of the plates.

Despite the fact that some inconsistencies exist, the observed relationship between counting methods is very good. Since the actual number of colonies on any given plate is in reality unknown, it is not possible to determine which method, in fact, estimates colony numbers more accurately. For this reason, one is led to consider variability as the major criterion of comparison. Furthermore, such variance cannot be interpreted as a common variance, but rather an average over some distribution of variabilities, the distribution depending on unknown characteristics of the plates other than actual colony count.

With the above considerations in mind it seems fair to suggest that the precision of the 3M counter is acceptable over the range of counts ordinarily considered readable (30-300) by the SPC method, even though within-plate reproducibility at very low counts is somewhat greater than 5%. If desired, plates in the range of 30-50 colonies could easily be read by the standard procedure, along with any spreader plates.

Acknowledgement

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EFFECT OF TYPE OF ASSAY FLASK CLOSURE ON THE GROWTH RESPONSE OF *SACCHAROMYCES CARLSBERGENSIS* TO VITAMIN B_6[1]

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Abstract

Studies were made to compare the effect of different flask closures (cotton, plastic foam, parafilm, and metal) on the growth response of *Saccharomycos carlsbergensis* (*sacch aromyces uvarum)* used to assay for vitamin B_6. The organism showed maximal growth response and gave optimal standard curves when the assay was done in cotton-plugged flasks.

The Food and Drug Administration has published food labeling regulations governing the nutritional content of food products *(11)*. The declaration of vitamin B_6 on the nutrient label will be mandatory if it is added. It may be listed when vitamin B_6 occurs naturally in the food product. The food samples are to be "analyzed by Association of Official Analytical Chemist (AOAC) methods where available or, if no AOAC method is available, by reliable and appropriate analytical procedure." This regulation is effective for all labels ordered after December 31, 1973, and is final after December 31, 1974. Therefore, factors affecting the accuracy and sensitivity of the vitamin B_6 assay method need to be elucidated.

Saccharomyces carlsbergensis (*Saccharomyces uvarum (6)* is noted for its growth response to the total vitamin B_6 group: pyridoxine, pyridoxal and pyridoxamine *(4)*. For this reason, it is the preferred assay organism. At present, the AOAC assay method for determination of total vitamin B_6 in food uses *S. carlsbergensis* 4228 (ATCC No. 9080) *(10)*. In this procedure, a sterile cheesecloth covers the assay tubes which have plastic caps with an 1/8-inch hole through the top for inoculation. Also, Haskel and Snell *(3)* mention only as a side remark the possible use of cotton plugs for the assay flasks.

In general microbiological work, it is known that the type of flask closure will affect the growth response of aerobic microorganisms by limiting the suppy of oxygen to the culture. In comparing the growth of *Bacillus megaterium,* an obligate aerobe, in cotton-stoppered flasks with that in open flasks, Schultz *(9)* found that the initial growth rates are similar, but at higher cell densities growth was restricted in the flasks with the cotton. Corman et al. *(2)* suggested that use of three layers of milk filter discs "Rapid-flo" (Johnson and Johnson Co., New Brunswick, N. J.) as a flask closure would be far superior to cotton plugs where maximum aeration efficiency in shake flasks is required. No information is available, however, on the efficacy of simple flask closures such as metal, plastic foam, and parafilm in comparison to cotton plugs in the vitamin B_6 microbiological assay. In this study the effect of these types of closures on the growth response of *S. carlsbergensis* 4228 to vitamin B_6 was determined.

Materials and Methods

The assay flask closures included plastic foam (diSPo^R plugs, Scientific Products, Evanston, Ill.), cotton (non-absorbent, University plugging cotton, Rock River Mfg. Corp., Janesville, Wisc.), PARAFILM "M"^{R} (American Can Co., Neenah, Wisc.) and metal (stainless steel culture tube closure, Bellco Glass, Inc., Vineland, N. J.). Regular Erlenmeyer flasks were used for all closures except the metal ones which require Bellco DeLong culture flasks. *S. carlsbergensis* 4228 (ATCC 9080) was used to assay vitamin B_6.

S. carlsbergensis was grown on Micro Assay Culture Agar (Difco) slants at 30 C for 24 h for three successive transfers before the first assay. The inoculum was prepared aseptically by washing the yeast cells from a 24-h slant with 10 ml of sterile saline (0.9% NaCl) and centrifuging the suspension in sterile tubes. The supernatant liquid was discarded. The cells were washed twice more with saline and finally resuspended in sterile saline to give a 73-76% transmittance at 640 nm, using a Beckman DB Spectrophotometer. This suspension was used as the inoculum.

In preparing the standard curves, freshly prepared pyridoxine-HCl solution (2 ng/ml) was pipetted into duplicate series of 50-ml flasks to give a range of 0.0 to 10.0 ng pyridoxine per flask. The volume was brought up to 5.0 ml with twice-distilled deionized water. Five milliliters of Pyridoxine Y Medium (Difco) were then added to each flask. Four different standard curves were prepared using cultures growing in flasks fitted with four different types of closures: cotton plug, plastic foam, metal caps, two lay-

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TABLE 1. THE EFFECT OF THE TYPE OF ASSAY FLASK CLOSURE ON THE VITAMIN B_6 CONTENT OF NONFAT DRIED MILK

Closure type	μg Vitamin B_6/g
Cotton	1.3
Metal	1.3
Parafilm	1.1
Plastic foam	1.2

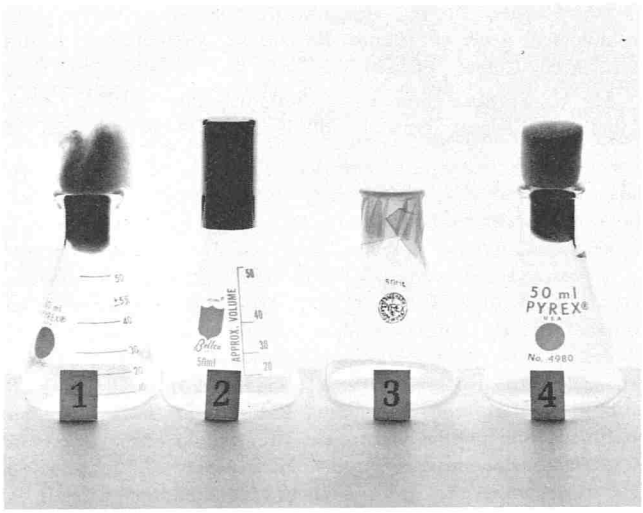

Figure 1. The four types of flask closures. 1. Cotton; 2. Metal cap; 3. Parafilm; 4. Plastic foam.

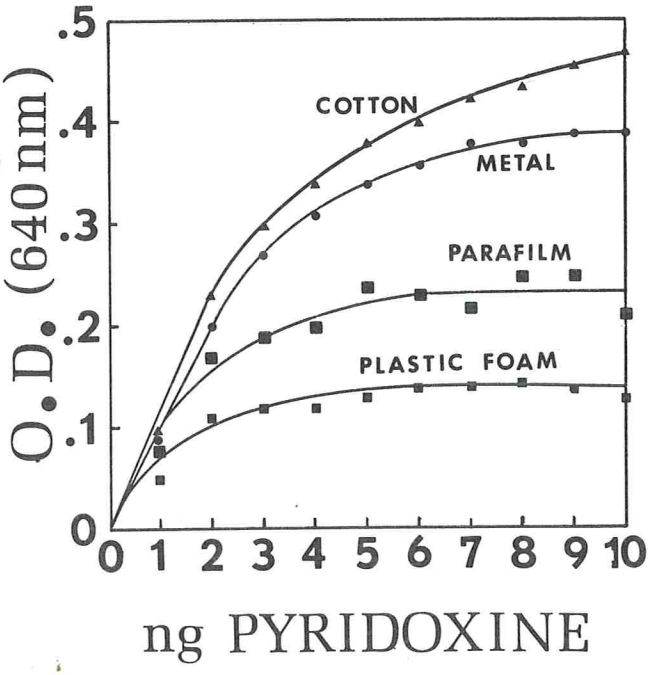

Figure 2. The effect of the type of assay flask closure on the growth response of *Saccharomyces carlsbergensis* 4228 to pyridoxine.

ers of Parafilm. All the flasks were autoclaved at 121 C for 10 min. cooled to room temperature, inoculated aseptically with one drop of the inoculum from a size 20 needle, and incubated at 30 C in a New Brunswick Incubator Shaker Reciprocating Modal R25) at 120 rpm for 18 h. The growth response in terms of optical density at 640 nm for each flask was recorded on a Beckman DB Spectrophotometer after correcting for the blank.

RESULTS AND DISCUSSION

Figure 1 illustrates the four types of stoppered flasks. Figure 2 shows the four standard curves obtained. Under the same experimental conditions, the cultures grown in cotton-plugged assay flasks showed the best growth densities and responded in growth over the entire range of 0 to 10 ng pyridoxine. Cultures grown in the metal-capped flasks responded only up to 6 ng pyridoxine, those in parafilm-covered flasks only up to 5 ng pyridoxine, and those in the plastic foam-stoppered only up to 3 ng pyridoxine.

As observed, the differences in the growth response of the yeast appear to be related to the extent of aeration provided by the different closures. The cotton plug evidently allowed for optimum air transfer to the yeast cells. These results are also in agreement with those of Morris et al. *(8)* who observed that alteration of the surface area:volume ratio in static cultures by variously sloping the assay tubes affected the aeration rate and response curves of *S. carlsbergensis*. Atkin et al. *(1)* achieved the most rapid growth of *S. carlsbergensis* when cultures were agitated constantly.

Furthermore, Ladd *(5)* noted that in stationary culture tubes the growth of *Pseudomonas flourescens* in the tubes covered with stainless steel closures attained only one-half that observed in tubes with cotton or foam plastic closures (Identi-plug, Gaymar Industries, Inc., Buffalo, N.Y.). With shaker studies of *Escherichia Coli*, McDaniel and Bailey *(7)* found no significant difference between absorbent cotton plugs and plastic foam plugs (Identi-Plugs, A. H. Thomas Co., Philadelphia, Pa.), but in this study, the cotton plugs were definitely superior to plastic foam plugs.

To ascertain what effect the type of closure would have on sample assays, we analyzed nonfat dried milk for vitamin B_6. When the B_6 content of the sample fell within the range of the standard curves, we found little change in vitamine B_6 values (Table 1). Thus, under the experimental conditions outlined here, the use of cotton-plugged shake flasks would provide a wider working range and would result in maximal growth response.

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A SELECTIVE PLATING AGAR FOR DIRECT ENUMERATION OF *SALMONELLA* IN ARTIFICIALLY CONTAMINATED DAIRY PRODUCTS[1]

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Abstract

A selective plating medium was developed that allows direct enumeration of salmonellae in dairy products such as nonfat dry milk, and Cheddar and cottage cheese. The agar medium developed was a modification of the Lysine-Iron-Cystine broth of Hargrove et al., 1971. Strains of species of the genera *Escherichia, Enterobacter, Citrobacter, Proteus, Shigella, Pseudomonas,* and *Bacillus* were easily differentiated from salmonellae by colony color and color of surrounding area or absence of growth. The antibiotic, novobiocin, used as a selective agent, inhibited growth of some *Proteus, Shigella,* and *Escherichia;* however the antibiotic was most effective against *Bacillus,* without having any observable effect on salmonellae. The medium was sufficiently sensitive and selective to permit detection of as few as 1-2 salmonellae per gram of product in the presence of naturally occurring bacteria and should be of considerable value in following the *Salmonella* content of artificially contaminated foods during processing and also during storage.

Several different types of bacteriological media have been proposed for isolation and identification of *Salmonella* in foods. Most of these were designed for selective preenrichment or for differential growth on surface agars. Methods for direct quantitative enumeration consist almost entirely of a most probable numbers procedure (MPN) *(6)*. Some workers contend that if dried foods are to be examined, preenrichment and quantitative estimation by MPN is essential *(2)* and perhaps the only way of estimating the degree of contamination. Sperber and Deibel recently reported an accelerated procedure for *Salmonella* detection in dried foods *(5)*. There appears to be a definite need for a selective direct plating procedure which would permit a more rapid and reliable estimation of *Salmonella* particularly with artificially contaminated foods. The advantages of direct plating of a food, is that as a research tool, one can follow the effect of processing and storage on the *Salmonella* bacteria even in the presence of other naturally occurring bacteria.

In a recent study reported by Hargrove et al. *(1)* a lysine-iron-cystine broth was used effectively for presumptive detection of *Salmonella* in dairy products and as a one-step preenrichment and selective medium in a 24-h immunofluorescent procedure for salmonellae in non-fat dry milk (NDM) which is capable of detecting one *Salmonella* per 100 g (4). The present study reports the adaptation of this medium as a selective plating agar medium for the direct enumeration of *Salmonella*.

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Materials and Methods

Pure cultures of salmonellae and related types were used in developing the plating medium; these included 36 *Salmonella* serotypes and representative strains of the species of the genera *Escherichia, Enterobacter, Citrobacter, Proteus, Shigella, Pseudomonas,* and *Bacillus.*

In preliminary screening tests several standard and selective agar media for *Salmonella* were compared in the direct enumeration of stock cultures of salmonellae in milk. Media compared were Bismuth Sulfite agar (Fisher), Brilliant Green agar (Difco), Salmonella-shigella agar (Baltimore Biological Laboratories), MacConkey agar (Difco), and a lysine iron agar prepared from Hargrove's lysine-iron-cystine broth *(1)*. Total plate counts were compared with those obtained with Standard Plate Count agar (Difco).

The type and degree of injury that normally occurs to *Salmonella* in processed foods should be closely simulated by the method of preparation of contaminated product. Organisms used were fresh 18 h cultures grown on trypticase soy broth. Dilutions of these were inoculated into pasteurized whole milk (Cheddar cheese) and/or skim milk (non-fat dry milk) before manufacturing into specific dairy products in our pilot plant. Fresh skim milk for Cottage cheese was fortified to 11% solids with NDM and pasteurized. Products tested include nonfat dry milk (NDM) and Cheddar and cottage cheese.

Medium

The lysine-iron-cystine broth medium as proposed by Hargrove et al. *(1)* was slightly modified for use as a plating medium. The significant changes were the substitution of brom cresol purple as a pH indicator and an increase of the cystine content from 0.1 to 0.2 g per liter. The medium which was formulated and which showed the greatest selectivity for all *Salmonella* strains is listed below:

L-Lysine, 10 g; Bacto tryptone, 5 g; yeast extract, 3 g; lactose, 5 g; salacin, 1 g; glucose, 1 g; L-cystine, 0.2 g; ferric ammonium citrate, 0.5 g; sodium thiosulfate, 0.5 g; brom cresol purple, 0.02 g; agar, 15 g; distilled water, 1000 ml. The medium was adjusted to pH 6.4-6.5, dispensed in flasks, and sterilized at 121 C for 15 min.

Novobiocin (Upjohn) was added aseptically to the cooled

TABLE 1. GROWTH OF REPRESENTATIVE PURE CULTURES IN SELECTIVE MEDIUM[1]

Organism	LICA growth	Colony appearance	Medium reaction
Salmonella cubana	+++	Black-lens	Alkaline-purple
Salmonella montevideo	+++	Black-lens	Alkaline-purple
Salmonella oranienburg	+++	Black-lens	Alkaline-purple
Salmonella tennessee	+++	Black-lens	Alkaline-purple
Salmonella worthington	+++	Black-lens	Alkaline-purple
Salmonella senftenberg	+++	Brown-lens	Alkaline-purple
Salmonella choleraesuis	+++	Brown-lens	Alkaline-purple
Salmonella paratyphi A	+	Black-lens	Purple
*Salmonella tennessee***	+++	Black-lens	Acid-yellow
Escherichia coli	±*	White	Acid-yellow
Proteus vulgaris	±	White	Slightly acid
Proteus morganii	–	–	–
Proteus mirabilis	+++	White	Acid-yellow
*Bacillus megaterium****	–	–	–
Bacillus cereus	–	–	–
Bacillus subtilis	–	–	–
Bacillus sterothermophilus	–	–	–
Aerobacter sp.	+++	White	Acid-yellow
Citrobacter sp.	+++	White	Acid-yellow
Pseudomonas sp.	±	White	Acid and alkaline
Shigella sp.	–	–	–

[1]The newly developed lysine-iron-cystine agar (LICA).
*± Some strains inhibited.
** Lactose positive.
*** Bacillus strains tested at 10^6 organism/plate.
+++ = 300-100 colonies corresponding with counts on Plate Count Agar of between 300 and 100 colonies.
+ = Few colonies 3-30, control plates were between 300-100 colonies.

(45 C) melted agar just before plating to give a final concentration of 5 μg/ml. As previously shown *(1) novobiocin* selectively inhibited growth of undesirable gram-positive microorganisms, particularly sporeformers. Selected strains of *Escherichia coli, Proteus, Pseudomonas,* and *Shigella* were also inhibited.

Trypsin (Nutritional Biochemical 1-3000) was added to the medium or plates containing .25 to 1 g of dairy product to digest milk casein *(3)*. Stock sterile trypsin solution was previously described *(1)* and usually 1-2 ml of the trypsin solution was added to the cooled melted agar medium.

Plating procedure

Because of the spreading growth of *Salmonella* in the medium, agar plates were poured in three stages.

An underlayer (10-15 ml of single strength agar) was added to sterile petri dishes and allowed to solidify. The sample dilution was added and poured with a second layer of agar (10-15 ml) and allowed to solidify. (Double strength agar was used for the second agar layer when the sample aliquot exceeded 1 ml). Plates were then covered with a third "sealing" layer of agar. Plates were incubated at 37 C for 24 h; only the black colonies were recorded, and then all plates were reincubated for an additional 24 h to detect slight H_2S producers.

NDM and cheese samples were prepared for plating as follows:

A 25% (W/V) milk concentrate was prepared from NDM powder by adding sterile distilled water (85 ml) to 25 g of powder. The mixture was blended in a sterile blender and the volume was adjusted to 100 ml before plating. A maximum of 4 ml of the concentrate could be added to a 100-mm diameter glass petri plate. The concentrate was used to make

TABLE 2. COMPARISON OF DIRECT PLATE COUNT[1] AND MPN FOR ESTIMATING SALMONELLAE IN NDM

NDM Lot. no.	Age (Weeks)	Contaminating *Salmonella*	*Salmonella*/g Direct count[2]	MPN
1 A	1	S. *newbrunswick*	320	460
	2	"	296	460
	3	"	220	240
	4	"	80	43
	6	"	39	43
1 B	1	S. *cubana*	480	460
	2	"	445	460
	3	"	310	240
	4	"	150	93
	6	"	80	93
1 C	1	S. *newbrunswick*	80	75
	2	"	34	43
	3	"	34	43
	4	"	30	43
	6	"	2	0
1 D	1	S. *cubana*	44	43
	2	"	39	43
	3	"	31	43
	4	"	9	9.1
	6	"	1	3.6

[1]Using the newly developed lysine iron cystine agar.
[2]Average value of triplicate platings of samples.

TABLE 3. ASSAY OF *Salmonella*-CONTAMINATED COTTAGE CHEESE DURING PROCESSING

Point in manufacture	Lysine-iron cystine count/ml-g	MPN[2]/ml-g
Cheese milk	21×10^4	24×10^4
Milk coagulated (before cutting)	25×10^4	24×10^4
Whey before cooking[1]	27×10^3	32×10^3
Whey before drain	0	0
Curd-uncreamed	0	0

[1]Cooked at 120 F for 20 min.
[2]5 tube MPN.

TABLE 4. ASSAY OF SALMONELLAE IN ARTIFICIALLY CONTAMINATED CHEDDAR CHEESE DURING MANUFACTURE AND STORAGE USING THE LYSINE-IRON-CYSTINE SELECTIVE PLATING AGAR

Time	Make status	Number of cells 11 ml-g
2 h 15 min.	Inoculated pasteurized milk	33×10^2
3 h 15 min.	Whey-end of cooking (100 F)	23×10^1
3 h 15 min.	Whey-drained	47×10^1
24 h	Curd-drained	60×10^4
2 wk	Begin (curing) storage at 50 F	54×10^4
6 wk	Curing 50 F	23×10^4
14 wk	"	19×10^4
18 wk	"	24×10^3
26 wk	"	11×10^3
30 wk	"	15×10^2
34 wk	"	2×10^1
0	"	0

the appropriate tenfold dilutions of the powder.

Eleven-gram samples of cheese were emulsified in 99 ml of sterile distilled water containing 0.2% sodium citrate. A maximum of 5 ml of the cheese slurry could be added per plate. Dilute solutions of milk or cheese required no trypsin.

The MPN *(6)* was determined in lactose broth. Selenite broth also was used occasionally to determine whether coliform overgrowth was an interfering factor. After incubation at 37 C for 18 h, 0.05 ml from each MPN tube was streaked onto Brilliant Green agar. Growth from typical colonies was transferred to Lysine agar (Difco) and Triple Sugar Iron agar (TSI; Difco). Growth from tubes exhibiting typical *Salmonella* reactions was checked for agglutination with type 0 polyvalent *Salmonella* antisera (Difco).

RESULTS AND DISCUSSION

Except for lysine-iron and MacConkey's agar, all of the test media used for comparative enumeration of *Salmonella* in milk showed some degree of inhibition. Nearly all *Salmonella* grew rapidly in the medium and formed large, black lenticular colonies in 24 to 48 h. Typical results from pure cultures are shown in Table 1. A deeper purple zone about the colonies indicated an alkaline reaction due to the action of lysine decarboxylase. Strains of *Salmonella choleraesuis, Salmonella sendai,* and *Salmonella senftenberg* produced brownish rather than typically black colonies in 48 h. These strains are known to produce little or no hydrogen sulfide on TSI agar. A lactose-positive *Salmonella tennessee* gave an acid medium reaction but the colony was the typical black. Coliforms and *Citrobacter* utilized lactose and salacin and gave an acid reaction (yellow) in the medium around their white lenticular colonies; colony growth remained white without blackening. All the *Bacillus* species used were very sensitive to 5 μg novobiocin/ml and as many as 10^7 cells/ml were inhibited. Additionally many gram-positive organisms were inhibited by 5 μg of novobiocin/ml, as was *Shigella,* some strains of *E. coli,* and species of *Proteus and Pseudomonas. Salmonella paratyphi A* showed slight inhibition with 5 μg of novobiocin/ml. Most salmonellae grew readily in the medium with levels of antibiotic as high at 10 μg/ml.

An agar underlayer was essential before addition of the sample to prevent colony spreading on the bottom of the plates. Similarly, after plates were poured an overlayer was essential to prevent surface spreading. Without an overlayer *Salmonella* colonies were indistinguishable from coliforms and other gram-negative organisms, because all surface colony growth turned black and gave an alkaline reaction in the medium.

Trypsin was essential to clarify the medium when milk concentrates of dairy products were plated *(4).* Casein digestion and medium clearing was usually complete after 4 to 6 h incubation. A comparison of the direct plate count and the three tube MPN (a prepared table estimated value) procedures *(6)* in repeated sampling of four lots of NDM during storage is shown in Table 2. These data show that the direct plate count is as sensitive as the MPN at these levels of contamination. The decline in viable cells over a 6-week period can be seen in both the high and low contamination level. The medium and plating procedure was sufficiently sensitive and selective to permit detection of as few as 1-2 salmonellae per gram of product. While reliability of results from such low counts may be questionable, they were generally reproducible. In following the manufacture of *Salmonella* contaminated cottage cheese one can see it can easily be monitored with lysine-iron-cystine agar (LICA), and the agar can be used effectively to establish that the cooking stage (or sanitizing step) is effective (Table 3).

Artificially contaminated Cheddar cheese can be analyzed for *Salmonella* with LICA during manufacture and curing (storage at 50 F). Note that multipli-

cation of *Salmonella* has occurred during manufacture (Table 4). It is believed that this medium will be of considerable value to researchers interested in following the progress of *Salmonella* in artificially contaminated food products.

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INFLUENCE OF THE HARVEST METHOD ON CONTAMINATION OF FRUIT BY *BYSSOCHLAMYS* ASCOSPORES

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Abstract

Mechanically-harvested tart cherries and Concord grapes were not more heavily contaminated with *Byssochlamys* ascospores than were hand-picked fruit. The data suggested, however, that both harvest procedures may be sources of contamination, probably because of soiled surfaces. Studies on various *Byssochlamys* isolates revealed marked differences in heat resistance.

Only in recent years has the heat-resistant mold, *Byssochlamys*, been recognized as a spoilage agent of canned fruits and fruit products in the United States (2). The present widespread use of machines to harvest many fruits raised the question as to whether changes in harvest procedures might have increased the opportunity for contamination by this organism. The objective of this study, therefore, was to compare the incidence of *Byssochlamys* spores on red tart cherries and Concord grapes that were picked by machine and by hand.

Materials and Methods

Samples

The New York-grown fruit was collected at the receiving platforms of area factories. Samples were sealed in polyethylene bags and held in an ice chest until placed in freezer storage at −23 C. In general, each sample represented material from a different grower. Water samples also were taken when cherries were transported in tanks containing chilled water.

The Michigan cherries were not commercially harvested but rather represented fruit that was picked under the supervision of quality control personnel. The fruit was placed in polyethylene bags, frozen, and then shipped to the Geneva laboratory over dry ice.

Enumeration

The method used to culture fruit for *Byssochlamys* ascospores has been described (3). Briefly, approximately 100 g of cherries or grapes were blended 5 min with 100 ml of 15° sterile Brix Concord grape juice. The blender jar with the blended material was then placed in a 70 C water bath for 2 h to destroy heat-sensitive organisms and to activate dormant ascospores. (Activation was enhanced by the Concord grape juice.) After heating, the entire homogenate was distributed into petri dishes, about 10 ml per plate. Equal volumes of potato dextrose agar (pH 3.5) containing 3% agar were then added to the plates and the material was mixed. Colonies were counted after an incubation of 5 days at 32 C.

The procedure used for water samples differed in that spores were first concentrated by centrifugation of 200 ml for 30 min at 10,400 × g in a Servall SS-3 centrifuge. The centrifuged sediment was resuspended in 5 ml of 15° Brix Concord juice, then heated 75 min at 70 C.

To confirm that molds which developed on plates were heat resistant, different colony types were transferred to culture tubes containing 5 ml of 15° Brix Concord juice, a medium that affords good ascospore formation. Following an incubation of 28 days at 32 C, culture homogenates were tested for thermal resistance by enumerating the survivors after heating 60 min at 70 and 85 C.

Results and Discussion

Cherries

During the 1972 season surveys were conducted at two cherry processing plants on four separate days. The results (Table 1) indicated that although *Byssochlamys* ascospores were not uncommon contaminants of tart cherries, their presence could not be correlated with the harvest procedure. Thus hand-harvested and mechanically-picked fruit gave comparable figures with respect to both spore counts and the percentage of *Byssochlamys*-positive samples.

Fruit collected directly from orchards under relatively "aseptic" conditions yielded a much lower incidence of *Byssochlamys;* the mold was recovered from only one sample. Although these cherries also differed from the factory-collected samples in that they were from the 1973 crop and were grown in

Table 1. Incidence of *Byssochlamys* on red tart cherries harvested by various procedures

Source	Harvest method	No. of samples	Percent positive	Spores/ 100 g
Factory 1[a]	Hand	14	100	23
	Machine	5	100	14
Factory 2[a]	Hand	11	55	4.9
	Machine	8	38	5.4
Orchard[b]	Hand, "aseptic"	25	4	1.0

[a]New York, 1972 season
[b]Michigan, 1973 season

TABLE 2. METHOD OF TRANSPORTING CHERRIES VERSUS THE LEVEL OF CONTAMINATION BY *Byssochlamys*

Sample	Number cultured	Percent positive	Spores per 100 g
Fruit shipped dry	14	100	21
Fruit shipped wet	24	58	9.8
Transport water	22	59	6.5[a]

[a]Spores per 100 ml

TABLE 3. *Byssochlamys* SPORES ON MECHANICALLY AND HAND HARVESTED CONCORD GRAPES

Sample	Number cultured	Percent positive	Spores per 100 g
Hand harvested	25	56	1.6
Machine harvested	16	56	3.7

TABLE 4. THERMAL RESISTANCE OF *Byssochlamys* CULTURES ISOLATED FROM RED TART CHERRIES

Isolate	Activated spores[a]	Heated 1 hr 85 C	
	Viable count	Viable count	% Survival
	(× 10^4)	(× 10^4)	
20	100	50	50
72b	100	44	44
21a	140	49	35
19a	200	47	24
6	100	24	24
1a	130	34	26
53	180	50	28
9a	95	30	32
72	120	20	17
109	100	26	26
4a	210	0.0055	0.0026
80	210	0.031	0.015
326	140	0.18	0.13
83	90	<0.001	<0.001
87	360	0.0085	0.0024
87a	84	<0.001	0.0012
87b	56	0.012	0.02
87c	33	0.0025	0.0076
4	13	<0.001	<0.0077
8	3.6	0.046	1.3
97	1.2	<0.001	<0.08

[a]Heated 1 h at 70 C (equals 100% survival)

Michigan, these differences probably do not explain the results. Rather, it is believed that the higher counts on the fruit collected at the factories reflect contamination that can occur during commercial harvest. Sources of this contamination include harvesting machines as well as soiled surfaces of containers such as lug boxes. Wooden boxes in particular are very difficult to clean. We might add that the idea of containers being a source of *Byssochlamys* is not new: it was observed in England over 40 years ago *(1)*.

Many growers transport their fruit to the factory in chilled water. The purpose is to prevent scald, brown spots that develop when cherries are bruised. Typically, one-half ton of fruit plus 60 gal of cool, 10 to 14 C, water are contained in a tank. It appears that this practice physically removes some of the ascospores from the fruit surface and consequently reduces the number that are brought into the processing plant. Thus a lower number of contamnated samples was found with the water-transported fruit and the number of ascospores per 100 g also was less (Table 2). With samples from two growers, the water yielded *Byssochlamys* while the cherries were negative. It is believed that the influence of the water-transport method explains the difference in spore loads found in fruit delivered to Factory 1 and Factory 2 (Table 1). Factory 2, a larger operation, received more of its fruit in the tanks.

Grapes

Grapes that were cultured represented fruit that was delivered to one of our larger processors over a 24-h period. The samples were from different vineyards. Results (Table 3) were similar to those obtained with sour cherries in that again the data did not incriminate mechanical harvesting as a source of *Byssochlamys* contamination. (The slightly higher average spore count on the machine-harvested fruit would not be statistically significant).

The finding that commercially-harvested Concord grapes yielded fewer spores than commercially-harvested sour cherries was unexpected since grapes are more susceptible to soil contamination, a significant source of *Byssochlamys* spores *(4)*.

Because grapes are a relatively perishable food, the juice industry has prescribed rigorous sanitation regimens—it is believed that this has reduced contamination by *Byssochlamys* as well as by yeasts and other molds. To illustrate: The processor who cooperated in this study used only plastic lug boxes for transport of hand-picked fruit; boxes were cleaned and sanitized at the factory. Operators of mechanical harvesters were given very detailed instructions for cleaning. Included as requirements were a hosing off of the harvester every 3 to 4 h and a thorough cleaning with a strong detergent-sanitizer at the end of each shift.

Heat resistance

Studies on thermal resistance of various isolates indicated considerable difference between cultures (Table 4); about 50% of the isolates exhibited rela-

tively high resistance in that 10% or more of the spore population survived a heat treatment of 1 h at 85 C while many of the others yielded survivals well below 1% when subjected to this treatment. As might be expected the number of spores present in the different homogenates had little bearing on the percent survival. Thus isolate 87 which had the highest spore count when activated at 70 C gave a survival figure of only 0.0024% when heated at 85 C for 1 h.

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Controlling food processing costs will be the theme of the sixth Food Engineering Forum Oct. 24, 1974, at Dallas.

Robert P. Graham, selected as recipient of the 1974 Food Engineering Award, will deliver the award address, "Process Modification to Avoid Pollution." Graham is research leader in food engineering applications for the U. S. Department of Agriculture's Western Regional Research Laboratory.

Dr. George E. P. Box, R. A. Fisher professor of statistics at the University of Wisconsin, will speak on cost control in the food industry. William P. Egar, group leader of instrumentation and computer applications of the Pillsbury Co., will deal with computer-assisted process control and the development of process control strategies.

The Forum is conducted biennially for the benefit of food processors attending Food and Dairy Expo, scheduled for Oct. 20-24. Time of the presentation is 9 to 11 a.m., Thursday, Oct. 24, at the Dallas Convention Hall. There is no registration fee. The Forum is sponsored by Dairy and Food Industries Supply Association and the American Society of Agricultural Engineers.

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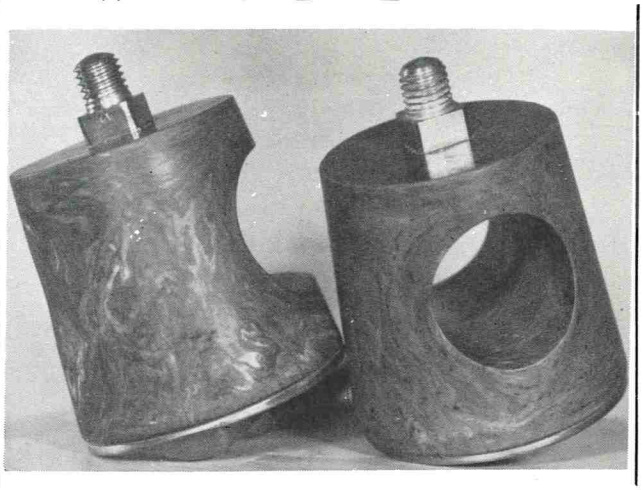

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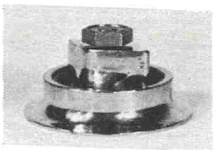

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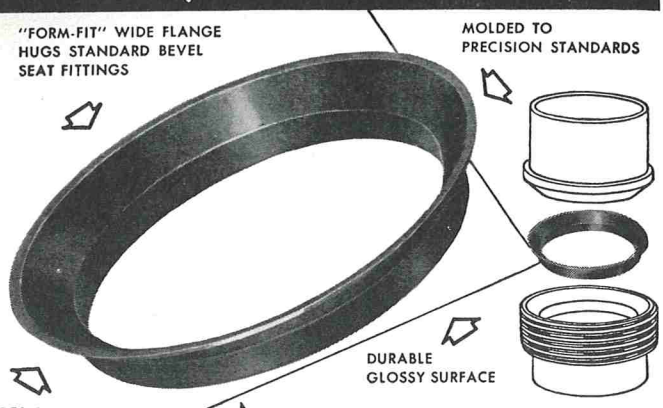

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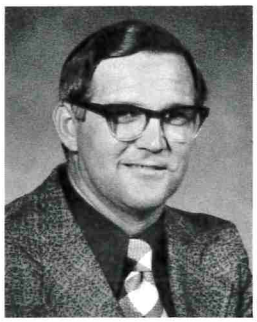
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

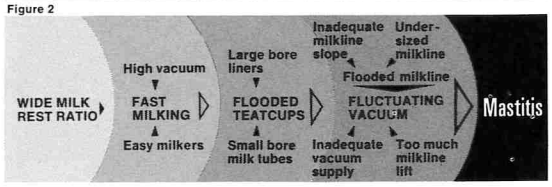
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

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This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.