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The Effect of Topically Applied Fluorides On Dental Caries Experience

VII. Consolidated report of findings for four study groups, showing reduction in new decay by individual tooth and by tooth surface, and frequency distribution of newly decayed teeth in treated and untreated mouth halves

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Previously reported studies in this series (*1–6*) have been concerned with the over-all effect of topically applied fluorides on dental caries experience in the permanent teeth of children. In summary, these studies indicate:

1. A series of four topical applications of a 2 percent solution of sodium fluoride, preceded by dental cleansing, effects a 40 percent reduction in dental caries incidence. More than four applications do not increase the caries-prophylactic effect.

2. The caries-inhibiting value of topically applied sodium fluoride is not appreciably decreased during a 3-year period following treatment.

3. The omission of dental cleansing prior to a series of applications reduces the effectiveness of the fluoride applications by approximately one-half.

4. Application of a saturated solution of lead fluoride (0.06 percent), using the same application technique as for solutions of sodium fluoride, is not associated with a significant reduction in the incidence of dental caries.

5. The application of a 2 percent solution of sodium fluoride to the teeth, followed immediately by a 5 percent solution of calcium chloride, does not increase the caries-prophylactic effect accomplished by the use of a solution of sodium fluoride alone.

6. An increase in the time interval between applications of the fluoride solution in a given series of applications from one or two a week to one each 3 to 6 months decreases the caries-prophylactic effect observed.

7. A concentration of 1 percent solution of sodium fluoride appears

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to be equally as effective in inhibiting new dental caries as a 2 percent solution.

In four groups previously studied (*1*, *5*, *6*) a series of 4 or more applications of sodium fluoride solution effected approximately a 40 percent reduction in caries incidence during a one-year study period. Inasmuch as none of these studies is based on a large enough sample of children to determine reductions in dental caries by individual tooth type or tooth surface, the data for the four groups have been combined. The purpose of this study is to present for different kinds of teeth, and by tooth surface, the reduction in new caries effected by topical fluoride applications. In addition, the distribution of caries experience in fluoride-treated and untreated mouth halves is compared in order to determine the variation in individuals of the caries-prophylactic effect of topical sodium fluoride. Identification of the study groups according to previous report and the age classification of children in each group are presented in table 1.

Table 1. *Age distribution of four groups of school children examined 1 year after a series of fluoride applications had been made to the teeth in half the mouth of each child*

Number of applications	All ages	Children by age at time of treatment								
		7	8	9	10	11	12	13	14	15
Group No. 1 [1]	288	10	18	26	41	39	50	44	50	10
Group No. 2 [2]	225	17	18	32	31	31	28	25	26	17
Group No. 3 [3]	259	35	30	26	40	40	31	25	27	5
Group No. 4 [4]	260	30	46	33	28	46	32	29	13	3
Total	1,032	92	112	117	140	156	141	123	116	35

[1] 8–15 applications, 2 percent NaF, following initial cleansing, Arlington, North Mankato, St. Louis Park, Minn. (*1*).
[2] 4 applications, 2 percent NaF, following initial cleansing, Miami County, Ohio (*5*).
[3] 6 applications, 2 percent NaF, following initial cleansing, Miami County, Ohio (*5*).
[4] 4 applications, 1 percent NaF, following initial cleansing, Miami County, Ohio (*6*).

In the first study group, teeth in the left side of the mouth were treated, while teeth in the right side of the mouth served as controls. In each of the three other study groups, approximately half of the children received treatment on teeth in the left side of the mouth and the other half on teeth in the right side of the mouth. Fine pumice paste and a motor driven rubber cup were used for cleansing the teeth. A detailed dental examination was made with plane mirror and explorer under artificial light and with compressed air available for use at the discretion of the examiner. The method of fluoride application consisted of isolating the teeth on the treated side with cotton rolls, drying with compressed air, and wetting the crown surfaces of the teeth with fluoride solution. The applied solution was allowed to dry in air for from 3 to 4 minutes; then the cotton rolls were removed and the child dismissed.

One year after the series of applications was begun, the children were re-examined. The examiner did not know which teeth had been

treated or which were untreated controls. Analysis of the data on caries experience is confined to the erupted permanent teeth present at the time of the initial examination and fluoride application.

New Caries Reduction in All Teeth

The caries experience during a study year in fluoride-treated and untreated teeth of children in the four study groups is shown in table 2. At the time of initial examination approximately the same number of noncarious or sound permanent teeth were available in treated and untreated mouth quadrants. During the year, among the 1,032 children under study, the percentage reduction in caries attack on fluoride-treated as compared with untreated teeth was 40.3 percent. For the four studies separately, the reduction ranged from 38.7 percent to 41.7 percent.

Table 2. *Dental caries experience during a 1-year study period in fluoride-treated and untreated teeth of 1,032 children*

Quadrants	Initially non-carious teeth	Teeth becoming carious during study period	Percent attacked by caries	Quadrants	Initially non-carious teeth	Teeth becoming carious during study period	Percent attacked by caries
Treated:				Untreated:			
Upper	3,466	239	6.9	Upper	3,492	414	11.9
Lower	3,964	176	4.4	Lower	3,968	281	7.1
Total	7,430	415	5.6	Total	7,460	695	9.3

New Caries Reduction by Tooth

The measurement of percentage reduction in new caries, by comparing treated with untreated teeth, is based on the bilaterally equal occurrence of dental caries in left and right mouth quadrants of large groups of children. This bilateral symmetry in caries experience is also characteristic of homologous teeth on opposite sides of the mouth. Reduction in new decay, by tooth, is measured therefore by comparing the increment of caries in specific treated teeth with that in corresponding homologous teeth which were untreated.

Inasmuch as the increment of dental caries in certain teeth, such as lower incisors, is relatively small during a single year, even in the number of children included in this study, it is desirable to indicate which of the figures presented here can be considered statistically significant. For the purposes of this report, a probability of 0.0227 or less that an observed difference in rate of decay between teeth treated and untreated is due to chance is considered statistically significant.

The caries experience in two pairs of upper teeth (central incisor

and cuspid) and in three pairs of lower teeth (central and lateral incisors, and cuspid) was relatively small, and the number of children in this study is not sufficiently large to establish that the observed caries reduction in these particular teeth was not due to chance (table 3). Less than 15 percent of total new decay occurring in upper teeth and only 7 percent of that occurring in lower teeth during a single study year affected these teeth.

Table 3. *Percent less initial caries attack in fluoride-treated than in untreated teeth of 1,032 children, by specific tooth*

Teeth	Mouth quadrants			Teeth	Mouth quadrants		
	Upper	Lower	Both		Upper	Lower	Both
Central incisor	22.7	44.4	26.4	First molar	*34.7	*22.2	*28.7
Lateral incisor	*50.7	12.5	*46.8	Second molar	*46.7	*49.5	*48.1
Cuspid	33.3	66.7	41.7				
First bicuspid	*46.8	*52.0	*48.6	All teeth	*42.3	*37.4	*40.3
Second bicuspid	*50.9	*34.7	*43.3				

*Statistically significant.

Among the upper teeth for which the sample size is large enough to demonstrate statistical significance in the reduction in new caries, the range in reduction in treated teeth was from 34.7 percent in first molars to 50.9 percent in second bicuspids. In upper second molars, first bicuspids, and lateral incisors the reductions were 46.7, 46.8, and 50.7 percent, respectively.

In upper central incisors, a 22.7 percent reduction in new caries in treated teeth was found. The probability that this or a greater reduction is due to chance is 0.1515, and therefore is not statistically significant. Inasmuch as the mesial surface of the untreated central incisor is almost certain to be wet in most cases when fluoride solution is applied to the same surface of the homologous tooth in the treated half of the mouth, the amount of reduction in new decay observed in this tooth must be analyzed on the basis of this condition. When the analysis is made separately for the observed increment of new caries on mesial and on distal surfaces of the upper central incisor, it is found that mesial surface decay is slightly greater in treated than in untreated teeth, while distal surface decay was reduced approximately 40 percent.

Among lower teeth, the fluoride applications effected the lowest significant reduction in initial caries attack in first molars—22.2 percent, and the highest in first bicuspids—52.0 percent. New caries in lower second bicuspids was reduced 34.7 percent and in second molars 49.5 percent.

The number of teeth classified as sound or noncarious on initial examination and the proportion that became carious during a study year, by tooth type, and by treated and untreated mouth quandrants are shown in table 4.

Table 4. *Number of initially noncarious teeth, and percent attacked by caries in fluoride-treated and untreated teeth of 1,032 children, by specific tooth*

Teeth	Mouth quadrants											
	Upper				Lower				Both			
	Initially noncarious teeth		Percent attacked by caries		Initially noncarious teeth		Percent attacked by caries		Initially noncarious teeth		Percent attacked by caries	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Central incisor	810	803	4.2	5.5	1,001	998	0.5	0.9	1,811	1,801	2.2	2.9
Lateral incisor	720	729	4.7	9.5	967	969	.7	.8	1,687	1,698	2.4	4.5
Cuspid	473	461	1.3	2.0	613	615	.2	.5	1,086	1,076	.6	1.1
First bicuspid	532	539	4.7	8.7	586	592	2.0	4.2	1,118	1,131	3.3	6.4
Second bicuspid	422	449	6.4	12.2	431	419	7.4	11.7	853	868	6.9	12.0
First molar	294	293	21.8	33.4	199	199	35.2	45.2	493	492	27.2	38.2
Second molar	215	218	22.7	42.2	167	176	29.2	55.1	382	394	25.7	48.0
All teeth	3,466	3,492	6.9	11.9	3,964	3,968	4.4	7.1	7,430	7,460	5.6	9.3

New Caries Reduction by Tooth Surface

The reduction in new caries in all surfaces of fluoride-treated sound teeth in upper mouth quadrants for children in the four study groups was 40.6 percent, and in lower mouth quadrants the reduction was 34.1 percent (table 5).

Table 5. *Percent less initial caries attack in tooth surfaces of fluoride-treated than in untreated teeth of 1,032 children, by specific tooth surface*

Tooth surfaces	Mouth quadrants			Tooth surfaces	Mouth quadrants		
	Upper	Lower	Both		Upper	Lower	Both
Occlusal	*42.4	*37.9	*40.2	Buccal and labial	38.0	*50.0	*48.1
Mesial	*41.0	0	*31.3				
Distal	*45.1	4.5	*35.5	All tooth surfaces	*40.6	*34.1	*37.9
Lingual	14.3	100.0	22.6				

*Statistically significant.

In upper teeth, the reduction in new carious surfaces associated with topical fluoride applications was slightly greater for distal surfaces (45.1 percent) than for occlusal surfaces (42.4 percent). On mesial surfaces the initial caries attack was reduced 41.0 percent. This high proportionate reduction in new decay on interproximal surfaces is of particular interest since the dental cleansing which precedes topical fluoride applications is not a complete dental prophylaxis and the interproximal surfaces are not cleansed as thoroughly as the more accessible surfaces.

In upper mouth quadrants, more than half of the total new caries, by surfaces, in both treated and untreated teeth were occlusal surface

caries. Approximately 40 percent of the total occurred on interproximal surfaces (mesial and distal). Buccal and labial surface caries was relatively negligible, and the 38.0 percent difference between treated and untreated for this surface classification is not statistically significant. A similar conclusion applies to the 14.3 percent reduction observed for the lingual surface.

In lower teeth, statistically significant reductions can be demonstrated only for new caries on occlusal surfaces—37.9 percent reduction, and on buccal and labial surfaces—50.0 percent reduction. More than three-quarters of total new decay in both treated and untreated teeth in lower mouth quadrants occurred on occlusal surfaces. Little or no reduction was noted for the mesial or distal surfaces of lower teeth. This latter finding is noteworthy since it may indicate a failure to wet these surfaces properly with the technique employed.

The detailed data on caries experience by tooth surface in treated and untreated mouth quadrants are shown in table 6.

Table 6. *Number of initially noncarious tooth surfaces, and percent attacked by caries in fluoride-treated and untreated teeth of 1,032 children, by specific tooth surface*

Tooth surfaces	Mouth quadrants											
	Upper				Lower				Both			
	Surfaces initially noncarious		Percent attacked by caries		Surfaces initially noncarious		Percent attacked by caries		Surfaces initially noncarious		Percent attacked by caries	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Occlusal	3,466	3,492	3.9	6.8	3,964	3,968	3.4	5.5	7,430	7,460	3.7	6.1
Mesial	3,466	3,492	1.8	3.0	3,964	3,968	.8	.7	7,430	7,460	1.2	1.8
Distal	3,466	3,492	1.1	2.0	2,964	3,968	.5	.6	7,430	7,460	.8	1.2
Lingual	3,466	3,492	.7	.8	3,964	3,968	.0	.1	7,430	7,460	.3	.4
Buccal and labial	3,466	3,492	.1	.2	3,964	3,968	.6	1.1	7,430	7,460	.4	.7
All tooth surfaces	17,330	17,460	1.5	2.6	19,820	19,840	1.1	1.6	37,150	37,300	1.3	2.1

Frequency Distribution of Newly Carious Teeth in Treated and Untreated Mouth Halves

In the foregoing sections the caries reduction in individual teeth and on separate classes of tooth surfaces has been presented. The purpose of this section is to study the variation in effectiveness of the topical fluoride procedure on the teeth of individual children in the study group. In making this analysis the number of newly carious teeth occurring in each treated and untreated half of the mouth of each child is determined and the results arrayed in a frequency distribution.

The frequency distribution of the number of newly carious teeth

within treated and untreated mouth halves for the 1,032 children in the four study groups is given in table 7. This distribution shows that the maximum number of carious teeth in the fluoride-treated side of the mouth is three and for the untreated half, five. Furthermore, the number of children with one, two, or three newly carious teeth in treated mouth halves is considerably less than those having equal numbers in untreated upper and lower mouth quadrants. On the other hand, the proportion of treated mouth halves in which no new decay occurred during the study year was substantially increased over the corresponding number of caries-free untreated mouth halves. In general, these findings indicate that the reduction in caries incidence effected by topical fluoride applications in a large group of children is the result of a fairly uniform lowering of the number of newly carious teeth each child would have had if the teeth had remained untreated.

Table 7. *Frequency distribution of number of initially carious teeth accrued during study year in treated and untreated mouth halves of 1,032 children*

Number of newly carious teeth	Number of mouth halves					
	Untreated		Treated		Theoretical*	
	Number	Percent	Number	Percent	Number	Percent
0	546	52.9	703	68.1	678	65.7
1	329	31.9	253	24.5	295	28.6
2	114	11.0	66	6.4	55	5.3
3	35	3.4	10	1.0	4	.4
4	6	.6				
5	2	.2				
Total	1,032	100.0	1,032	100.0	1,032	100.0

*Assuming newly carious teeth in untreated mouth halves reduced by 40 percent.

This conclusion can be subjected to further examination by arbitrarily applying a 40-percent reduction to the caries experience observed in the control or untreated teeth of each child, arraying the results in a frequency distribution, and comparing this theoretical distribution with that observed for treated mouth halves. Under this method all children who actually experienced five newly carious teeth in the untreated group would be expected to have only three newly carious teeth had they been treated. Those actually experiencing four newly carious teeth would have two in most cases, but some would have three in order that the average be a 40-percent reduction. The resulting theoretical distribution is also presented in table 7. Comparison of the percent of mouth halves in each class according to number of newly carious teeth reveals a striking similarity between the treated, or observed, and the theoretical. Thus, this result supports the general conclusion that a series of four fluoride applications reduces dental caries incidence approximately 40-percent and that all children treated benefit to this extent.

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Summary

Previously presented data relating to the reduced incidence of dental caries in fluoride-treated as compared with untreated permanent teeth of 1,032 children have been presented and analyzed separately for each tooth type and tooth surface. In summary, the analysis indicates that:

1. For the study group included in this presentation, the over-all reduction in newly carious teeth in fluoride-treated as compared with untreated teeth was 40.3 percent—42.3 percent for teeth in upper mouth quadrants and 37.4 percent for teeth in lower mouth quadrants.

2. Among teeth in upper mouth quadrants, except central incisors and cuspids, the reductions varied from 34.7 percent for first molars to 50.9 percent for second bicuspids.

3. Among teeth in lower mouth quadrants, except incisors and cuspids, the reductions varied from 22.2 percent for first molars to 52 percent for first bicuspids.

4. The over-all reduction in newly carious tooth surfaces in fluoride-treated as compared with untreated teeth averaged 37.9 percent—40.6 percent in upper mouth quadrants, and 34.1 percent in lower mouth quadrants.

5. For upper teeth alone, the reduction in new decay on distal surfaces exceeded that on occlusal surfaces (45.1 percent on distal; 42.4 percent on occlusal), while mesial surface decay was lowered as the result of fluoride applications by 41.0 percent.

6. Comparison of the distribution of the number of newly carious teeth in treated and untreated mouth halves and a theoretical distribution calculated by applying a 40-percent reduction to the number of carious teeth observed in each untreated mouth half indicates that the caries prophylactic effect of topical sodium fluoride is remarkably uniform for individual children.

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Operation Studies of Home Milk Pasteurizers

By Robert C. Thomas, M. S.*

To protect the health of rural families without access to a commercially pasteurized milk supply, there has been a demand in the past few years for a milk pasteurization unit in the home. This is especially true where the problem of eradication of *Brucella* infection in cattle has impressed upon the minds of the people the dangers of the spread of this infection to the consumers of milk obtained from infected animals. To meet the demand for a method of heating milk in the home, the Public Health Service in 1934 (*2*) recommended that the milk be placed in an aluminum vessel on a hot flame and stirred constantly until it is heated to 155° F., then immediately setting the vessel in cold water and continuing stirring until cool. In 1940 (*2*), following some research by the Public Health Service on heating milk inoculated with a test organism (*4*), the recommended temperature was increased to 165° F.

Trout, Devereux, and Bryan in 1943 (*10*) reported experiments using the following methods of heating: (*a*) double boiler, starting either with cold or (*b*) with vigorously boiling water; (*c*) direct heat; and (*d*) in-the-bottle. Part of the summary and conclusions of this article was: "A safe, adequately pasteurized milk can be produced by heating one or two quart quantities of milk for 10 minutes in a covered double boiler containing one quart of vigorously boiling water." In 1945 (*1*) the University of Minnesota published a circular on the use of direct heat and the double boiler method for heating milk in the home. Schaenzer and Shiozawa in 1946 (*5*) described several methods of pasteurization for small retail dairies and also described three units classified as home milk pasteurizers.

With the extension of electric service into rural areas, several manufacturers and the Rural Electrification Administration of the U. S. Department of Agriculture became interested in the development of an automatic electric home milk pasteurizer. This type of equipment also is popular with families who have consumed pasteurized milk in the city and spend their vacations in the rural lake sections of the country where a pasteurized milk supply may not be available. In 1947 Trout and Bortree (*9*) reported on three types of home electric milk pasteurizers. They gave certain precautions for operation of these units, such as (1) operation for a longer period of time than that

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suggested by the manufacturer; (2) allowing sufficient head room for milk expansion upon heating in order to guard against contamination by bacteria growing in milk that may ooze out from the top of the container; (3) care of control of the water level in the in-the-bottle unit to prevent cooling water from being drawn into the bottles.

All of the above work was based largely upon reducing the number of bacteria and the inactivation of phosphatase in the main body of the milk. The fundamental principle back of the modern ordinance requirement for heating the air above the milk surface in batch pasteurizers was not considered.

Commercial Pasteurization

As these automatic units reached the market, health departments throughout the country received requests for information on the effectiveness of the heat treatment of milk by the different units. Such requests were referred to this laboratory and a study was started on the various units available at that time. Because a number of these requests were from people who wished to sell pasteurized milk, the units were first studied from the standpoint of the following definition of pasteurized milk as given in the 1939 Milk Ordinance and Code recommended by the Public Health Service (*3*). "The term 'pasteurization, pasteurized' and similar terms shall be taken to refer to the process of heating every particle of milk or milk products to at least 143° F., and holding at such temperature for at least 30 minutes, or to at least 160° F., and holding at such temperature for at least 15 seconds, in approved and properly operated equipment; Provided, That nothing contained in this definition shall be construed as disbarring any other process which has been demonstrated to be equally efficient, and is approved by the State health authority." Specifications included in the code are: (*a*) thermometers; (*b*) construction and operation to insure that the required pasteurization temperature and time will be applied to every particle of milk or milk products; (*c*) proper inlet, outlet valves and connections.

Due to the absence of (1) mechanical agitation of the milk in some units, (2) provision for auxiliary heating of the air above the milk surface, and (3) thermometers as part of the standard equipment, none of the units submitted for test achieved a product that complied with the above definition for pasteurized milk, and should not be accepted for commercial pasteurization in communities where the Public Health Service recommended pasteurization standards or their equivalent are in effect. In considering the cooling and bottling requirements that are necessary in connection with commercially pasteurized products, it is possible that, with further improvements, the in-bottle type

of pasteurizer might be developed to meet these requirements. Further consideration is given here to these units for use as home pasteurizers and not for commercial purposes.

Method of Study

In checking their performance, these units were operated as received with the exception of unit A, which was operated at several different heating water temperatures. Temperatures were checked with thermocouples placed at several points in the pasteurizer. Phosphatase tests were made on the main body of milk and on milk swabbed from the milk container above the milk surface line. In the phosphatase test made to check the heat treatment of milk on the container above the milk surface line, cotton on a glass applicator was used to swab milk from this surface. Glass was used in place of wood because false positive tests were obtained in some cases where the wood applicators were sterilized. This was demonstrated by positive tests in control swabs. All controls with glass applicators were negative. The amount of material picked up on the swab varied from 0.03 gram to 0.1 gram. In making the phosphatase test, the material on the swab was shaken in three ml. of boiled milk in order to obtain enough material for check tests. Because of the high dilution and the small amount of milk on the swab, a phosphatase test of less than one unit, along with thermocouple tests indicating that the surface and the air above the milk reached a temperature of at least 143° F. for the proper length of time, was considered necessary as indication of proper heat treatment.

Description of Units Studied

At the time that this study was started, there were at least four so-called home pasteurizers on the market. They could be divided into the following two types on the basis of the method of applying heat to the milk:

1. Units A, B, and C where the milk in an inner container was heated by hot water in an outer container. The water was heated directly over a hot plate (unit B) or by an immersion heater (units A and C.)
2. Unit D where the milk container was heated directly over a hot plate.

Test Results

Unit A—Milk Container Heated by Water Bath

Preliminary tests on this unit demonstrated that with the thermostat set for a temperature of 147° F. as recommended by the manu-

facturers, a period of about 37 minutes was required to heat the milk from 60° F. to 143° F. With an additional holding period of 30 minutes, the milk reached a temperature of about 146° F. or about one degree below the temperature of the heating water.

When operated under these conditions of time and temperature, a negative phosphatase test was obtained on the main body of milk. Thermocouple tests, however, indicated that the inner rim of the milk container above the milk surface line was exposed to outside air and did not receive sufficient heat treatment. Insufficient heat treatment was also demonstrated by obtaining a positive phosphatase test on milk swabbed from the inner rim of the milk container.

In a previous study reported by Thomas (*8*) using the unit A home milk pasteurizer with heating water at 147° F., coliform organisms (present in the raw milk as received) were reduced from a plate count of 580,000 per ml. to zero per ml. within 44 minutes when the milk reached a temperature of 143° F. The phosphatase in the main body of the milk was reduced to less than 2 Scharer units per ml. at 59 minutes when the milk had reached a temperature of 145.2° F. and had been held between 143° F. and 145.2° F. for 15 minutes.

Operating this unit at water temperatures of from 163° to 165° F. resulted in negative phosphatase tests on a swab sample by the New York City Laboratory Method I, but gave a positive test by the more sensitive New York City Laboratory Method II (*6*). Final rim temperatures were from 161.6° to 163.3° F., whereas air temperatures above the milk were irregular from 149° to 158.3° F. depending on outside disturbances which affected the circulation of air above the unit. Tables 1 and 2 illustrate typical tests showing temperatures obtained with thermocouples and the results of phosphatase tests

Table 1. *Temperature at various locations and phosphatase test on milk in automatic electric home pasteurizer—unit A set at 154.8° F. (typical test)*

Heating time (minutes)	Temperature and location of thermocouple				Phosphatase test Scharer units (N. Y. C. methods)	
	Air below cover (° F.)	Rim (° F.)	Milk (° F.)	Heating water (° F.)	I	II
0	----	----	50.0	151.0	>3.5	>3.5
9	100.6	102.3	110.6	134.3	----	----
16	112.0	123.6	126.0	142.0	----	----
21	118.6	131.6	135.0	150.6	----	----
27	132.6	138.3	143.0	152.0	----	----
30	130.3	141.3	144.6	150.6	----	----
36	134.3	147.3	151.3	155.3	----	----
44	151.3	149.3	153.0	155.3	----	----
56	135.0	151.3	154.0	155.3	----	----
69	142.0	152.3	154.0	155.3	----	----
Cooled 71	160.0	87.6	80.6	70.0	0	0
Swab of surface of container above milk	----	----	----	----	1.0	>2.0

upon the main body of the milk and the milk swabbed from the milk vessel surface.

Table 2. *Temperature at various locations and phosphatase test on milk in automatic electric home pasteurizer—unit A set at 165° F. (typical test)*

Heating time (minutes)	Temperature and location of thermocouple				Phosphatase test Scharer units (N. Y. C. methods)	
	Air below cover (° F.)	Rim (° F.)	Milk (° F.)	Heating water (° F.)	I	II
0	98.2	96.4	77.7	142.7		
10	118.6	119.3	122.4	142.2		
20	133.7	140.5	142.2	156.6		
30	146.3	153.5	155.3	161.6		
40	152.6	159.4	160.7	163.0		
50	154.8	161.2	162.0	164.3		
60	155.8	161.2	162.5	164.1		
Cooled 72	93.2	78.8	68.0	64.4	0	0
Swab of inner surface of container above milk					0	<2.0

Unit B—Milk Container Heated by Water Bath

This unit heated milk from 57° to 161.3° F. in about 43 minutes. The temperature reached 143° in about 37 minutes. The air above the milk reached a maximum temperature of 157.6° F. The phosphatase test on the main body of the milk was negative at this temperature of operation but milk swabbed from the cover seat was strongly positive to the phosphatase test and also gave a positive coliform test. The maximum temperature recorded by thermocouples placed in the cover seat depression was 158.9° F. and with the positive tests indicated that momentary heating to this temperature was insufficient

Table 3. *Temperature at various locations and phosphatase test on milk in automatic electric home pasteurizer—unit B (typical test)*

Heating time (minutes)	Temperature and location of thermocouple				Phosphatase test—Scharer units (N. Y. C. method)	
	Air below cover (° F.)	In milk		Heating water (° F.)	I	II
		Surface (° F.)	Bottom (° F.)			
0	62.6		58.0	59.0		
9	68.6	65.3	63.9	77.3		
27	107.6		105.6	121.0		
31	118.0		125.0	139.3		
33	127.0		131.0			
37			143.0	154.6		
43 [1]	157.3	161.3	161.3	172.3		
Cooled 64			80.6	73.0	0	0
Swab of cover seat (coliform +)					<3.5	>3.5

[1] Buzzer indicating end of pasteurization.

heat treatment. Table 3 provides a typical record of the temperature and phosphatase tests obtained in the operation of this unit.

Unit C—Milk in Bottles Heated by Water Bath

The thermoregulator in the unit received by us was set to control the water temperature at 148° F. The instructions received with the unit stated that the thermostat in the control bottle was set to activate the timer when the water in the control bottle reached a temperature of 143° F. In actual operation, however, the timer started when the water in the control bottle reached a temperature of 137.7° to 141.4° F. The time required to reach this temperature varied from 57 to 60 minutes. The final temperature at the start of the cooling period was from 146° to 147° F. There is no mechanical agitation of the milk in the bottles and the temperature in a single bottle varied from 138.6° to 142.6° F. at the start of the timer. At the end of the holding period of 31 minutes, the temperature varied from 146.5° to 147.2° F. The temperature of the air under the cap was 141.8° F. at the start of the timer, and 144.5° F. at the end of the holding period.

In spite of the low starting temperature, all phosphatase tests on the main body of milk were negative. This was probably due to the fact that the final temperature was above 143° F. No positive phosphatase tests were obtained on the small amount of milk that could be swabbed from the bottle surface or cap seat. In these tests the 512–516 gram weight cylindrical milk bottles were used. They were filled to within 1 inch of the top with cold milk and capped with lip cover caps. The water in the bath came up to the bottom of the

Table 4. *Temperature at various locations and phosphatase tests on milk in automatic electric home pasteurizer—unit C in-bottle (typical test)*

Heating time (minutes)	Temperature and location of thermocouples					Phosphatase test—Scharer units (N. Y. C. method)	
	Control bottle (°F.)	Air below cap (°F.)	In milk				
			Top (°F.)	Middle (°F.)	Bottom (°F.)	I	II
0	59.9	63.0	56.7	57.8	51.3		
10	67.1	73.8	68.5	60.4	62.1		
20	82.8	90.5	86.9	76.6	77.4		
30	100.0	106.3	104.0	91.0	95.4		
40	118.6	122.0	119.8	112.1	113.0		
50	123.3	133.3	133.7	129.4	129.7		
58 [1]	141.3	141.8	142.6	138.6	138.6		
60	142.9	142.9	143.8	140.4	140.2		
70	145.8	144.0	145.8	144.0	144.0		
80	146.7	144.3	146.3	145.4	145.8		
89	147.6	144.5	147.2	146.5	147.2		
Cooled 107	75.2	62.6	73.4	69.8	60.8	0	0
Swab of inner surface of milk container						0	0

[1] Timer indicating start of holding period.

rolled rim of the bottle and about ⅜-inch below the cap seat. Table 4 shows that there is a variation in the temperature of the milk in different locations in an individual bottle. From a safety standpoint it would seem advisable to raise the water temperature, to set the timer control to start when the lowest milk temperature is at least 143° F., and control the inflow of cooling water so that it does not cover the top of the bottles unless they are capped with pressure-tight nonporous caps through which water cannot enter the milk during cooling. Table 4 is a typical record of temperatures recorded by one thermocouple in the control bottle and thermocouples at four points in a single bottle of milk, and of phosphatase tests.

Unit D—Milk Container Heated Directly

In this unit the timer started at from 45 to 56 minutes after the heat was turned on. Initial milk temperatures varied from 40° to 50° F. The period from the start of the timer until the buzzer sounded varied from 40 to 42 minutes. The air temperature above the milk surface below the cover at the start of the timer varied from 127° to 133° F. and the milk temperature varied from 136° to 143° F. The final air temperature was 147.3° F. with milk temperatures leveling off at 151.3° to 154° F. Occasional positive phosphatase tests were obtained on milk swabbed from the top inside rim when milk came in contact with the cover at the start of operation. Table 5 is a typical record of the temperatures recorded by thermocouples placed in the air under the cover and at two points in the milk. It also shows the results of phosphatase tests.

Table 5. *Temperature at various locations and phosphatase test on milk in automatic electric home pasteurizer—unit D (typical test)*

Heating time (minutes)	Temperature and location of thermocouple			Phosphatase test—Scharer units (N. Y. C. method)	
	Air below cover (°F.)	In milk			
		Surface (°F.)	Bottom (°F.)	I	II
0			43.0		
17	73.3	76.3	78.0		
33	94.0	93.0	101.0		
49	126.0	128.3	136.0		
53 [1]	132.8	136.0	143.0		
63	141.0	145.3	151.3		
79	146.3	149.6	153.6		
92 [2]	147.3	151.3	154.0	0	0
Swab of top edge of pail				<2	>3.5

[1] Timer indicating start of holding period.
[2] Buzzer indicating end of pasteurization.

Other Methods of Heating Milk

Tests were also made on other recognized methods of heating milk in the home. These consisted of the use of an open aluminum vessel, a closed aluminum vessel and an aluminum double boiler. The milk container was the same inner container of the double boiler in all cases. This container was of about 2 quarts capacity and was filled only to the 1.5-quart mark in all tests. In the first test with the open aluminum container over direct heat and with stirring, 14 minutes were required to bring the temperature of the milk from 73° F. to between 163.4° and 167.8° F. After cooling by placing the vessel in cold water, a positive phosphatase test was obtained on the milk swabbed from the inner edge of the milk vessel. The positive phosphatase test indicates that the milk that comes in contact with the side of the vessel does not receive the same heat treatment as the main body of the milk. Table 6 shows the results obtained in a typical test by this method.

Table 6. *Temperature at various locations and phosphatase tests on milk heated in an open saucepan—direct heat, milk stirred (typical test)*

Heating time (minutes)	Temperature and location of thermocouples				Phosphatase test—Scharer units (N. Y. C. method)	
	Air above milk (° F.)	Pan rim above milk (° F.)	In milk			
			Surface (° F.)	Bottom (° F.)	I	II
0	66.2	59.0	62.6	58.1	.....	.....
2	73.0	69.8	72.9	72.5	.....	.....
4	79.2	84.2	90.1	88.7	.....	.....
6	87.8	93.6	105.8	108.0	.....	.....
8	89.6	115.2	120.2	122.9	.....	.....
10	96.8	131.4	137.8	140.9	.....	.....
12	111.2	.....	152.6	154.4	.....	.....
14	113.9	.....	163.4	167.9	.....	.....
Cooled 20	65.3	.....	82.3	82.3	0	0
Swab of inner surface above milk	.....	.....	.....	.....	>2.0	>3.5

In order to observe the effect of a covered vessel at approximately the same temperature, the same container was used. After 18 minutes of heating, the temperature of the air under the cover was 154.8° F., the temperature of the rim was 158.4° F., and the milk temperature varied from 161.6° to 168.4° F. The phosphatase test on the main body of milk was negative, but the milk swabbed from the inner rim and top edge was positive by the N. Y. C. Method II, although to a lesser extent than in the open vessel test. Table 7 shows the results obtained in this test.

The method suggested by Trout (*10*) was used. Here the water in the bottom of the double boiler was brought to vigorous boiling and the milk container was placed in the outer container with the same heat

Table 7. *Temperature at various locations and phosphatase tests on milk heated in a covered saucepan—direct heat, no agitation (typical test)*

Heating time (minutes)	Temperature and location of thermocouples					Phosphatase test—Scharer units (N. Y. C. method)	
	Air above milk (° F.)	Pan rim above milk (° F.)	In milk				
			Bottom (° F.)	Center (° F.)	Top (° F.)	I	II
0	77.0	51.4	42.4	40.1	53.6		
4	65.8	55.0	52.2	53.2	49.1		
6	67.3	63.7	66.2	68.4	59.5		
8	72.0	73.8	77.9	78.1	69.4		
10	84.2	91.9	98.2	99.5	90.5		
12	100.4	110.1	114.8	116.2	113.0		
14	121.6	129.9	137.5	137.1	126.0		
16	140.9	148.5	156.9	159.8	148.6		
18	154.8	158.4	167.5	168.4	161.6		
Cooled 28	75.2	69.8	75.2	88.5	85.6	0	0
Swab of inner rim of container						<1	>2

applied to the outer vessel. In 10 minutes the temperature of the milk in the closed vessel varied from 168.8° to 192.6° F. and the air temperature below the cover was 178.7° F. There was a wide variation in the temperature of the milk because of the absence of agitation, but the lowest milk temperature was well above the temperature necessary to destroy phosphatase. Phosphatase tests on the main body of milk and on material swabbed from the inner side and edge of the vessel were all negative. Table 8 is a typical record of temperatures and phosphatase tests when using the double-boiler method of heating milk.

Table 8. *Temperature at various locations and phosphatase tests on milk in a double boiler—covered, over constantly boiling water, no agitation (typical test)*

Heating time (minutes)	Temperature and location of thermocouples				Phosphatase test—(Scharer units (N.Y.C. method)	
	Air below cover (° F.)	In milk				
		Bottom (° F.)	Center (° F.)	Top (° F.)	I	II
0	63.5	43.7	42.4	41.9		
2	72.5	73.4	52.7	59.0		
4	96.8	95.4	78.8	93.2		
6	124.2	130.6	113.9	125.2		
8	150.8	166.8	140.0	153.1		
10	178.7	192.6	168.8	178.7		
Cooled 18	95.0	60.8	72.5	93.2	0	0
Swab of inner surface above milk surface					0	0

Discussion

On the basis of the phosphatase test and thermocouple record of the minimum temperature obtained, the double boiler method proved to be satisfactory. Heating the milk to about 165° F. in an open or

closed vessel by direct heat did not produce a high enough temperature of the air or vessel surface above the milk surface line to inactivate phosphatase in milk that might contaminate this surface. There was, however, less phosphatase in the swabbed sample of milk from the closed vessel. In open vessels higher temperatures are required to compensate for the variation in temperature between the main body of milk and that milk contaminating the vessel above the milk surface line. In areas of higher elevation it may be necessary to hold the milk over boiling water for a longer period of time to compensate for the lower mean boiling point of water at higher elevations. Stewart (7) published a table showing the mean boiling point of water at 5,000 feet to be 203° F., and at 15,000 feet to be 187° F.

With the home pasteurization units it is important to operate at temperatures sufficiently high to insure that the air and vessel surface above the milk surface line attains the desired temperature for the proper length of time. The positive phosphatase tests obtained on these surfaces, along with thermocouple recorded temperatures, show that this temperature-time combination is not always obtained by all of the home milk pasteurizers tested in this study.

In one experiment a positive phosphatase test was obtained on material swabbed from the vessel surface above the milk surface line when the thermocouple recorded temperature indicated heat treatment sufficient to reduce the phosphatase in the main body of the milk (table 2). This suggests that further study is needed on the relationship between temperature and phosphatase destruction in milk on this surface. The air temperatures recorded in table 2 are slightly higher than the milk temperatures in table 1, whereas the phosphatase test on the milk in table 1 is zero and the phosphatase test on material swabbed from the vessel surface in table 2 is slightly less than 2 units. A question arises relative to the possible requirement of higher temperatures for destruction of phosphatase in milk exposed to air or on surfaces above the milk surface line.

A study of the air and rim temperatures above the milk surface line in the units that have a definite holding period close to 30 minutes suggests a different time-temperature relationship for the destruction of phosphatase in swab samples from the different units.

Summary

The simplest method of satisfactorily heating milk in the home, to make it safe for consumption as fluid milk, is by the double boiler method. The temperature of heating, however, is not as well controlled; there is some precipitation of milk solids, and the milk may have a slightly cooked flavor.

Some of the automatic units should be operated at a higher temperature than has been suggested in the early literature. There should be some way in which thermometers could be inserted into the milk so that the temperature can be checked. Most manufacturers of this equipment suggest that temperatures be checked with thermometers. This necessitates elevating the cover of the unit which has no opening for the thermometer, thus lowering the temperature of the air above the milk, and the inner rim of the vessel above the milk line.

A negative phosphatase test on the main body of the milk does not necessarily mean that the milk that may come in contact with the vessel rim above the milk surface line will also show a negative test. As demonstrated in unit B, table 3, this milk might contain living coliform organisms, even though those contained in the main body of the milk are killed.

As the minimum limits of time and temperature combinations for the pasteurization of milk are approached, more refinements in equipment construction and more accurate controls are needed to insure that all milk is held for the correct time at the proper temperature.

When the process is conducted on the same basis as that of heating other foods in the home, where higher temperatures are involved, simpler equipment such as the double boiler can be used.

The use of the phosphatase test on material swabbed from the inner milk vessel surface above the milk level line is suggested as a check on proper heat treatment of milk that comes in contact with this surface during the pasteurization process.

Two solutions to the problem created by the absence of auxiliary methods for additional heating of the air above the milk level are:

1. Allow the heating water to completely surround the milk container. This will require tight-fitting nonporous covers that will not allow cooling water to enter the milk container.

2. Use higher temperatures which will insure that the milk vessel surface and the air above the milk level line will attain the proper temperature.

The manufacturers of units A and D have changed the construction of the tops of these units in order to raise the air temperature with the hope of overcoming the presence of active phosphatase in the milk swabbed from the vessel surfaces above the milk surface line. These units do not comply with the standards for commercial pasteurization, but when operated at temperatures that will insure proper heat treatment of the surface above the milk surface line, they compare favorably with the simpler double boiler methods of home milk pasteurization.

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Histoplasmosis in Rats and Skunks in Georgia

By C. W. Emmons, Ph. D., H. B. Morlan, B. S., and E. L. Hill, M. D.*

The occurrence of histoplasmosis in wild rats was first proved by the recent isolation of *Histoplasma capsulatum* from 10 brown rats (*Rattus norvegicus*) trapped in Loudoun County, Virginia (*3*). Subsequent studies (*2*) have brought the number of proved cases of murine histoplasmosis in that area to 27 and have indicated that the addition of the rat to the list of proved natural hosts of *Histoplasma* is of particular interest.

The significance of the rat in the epidemiology of histoplasmosis is not yet apparent, but the knowledge that it is a host of *Histoplasma* provides a useful tool for determining the geographic distribution of histoplasmosis. The rat is widely distributed over the earth, it is closely associated with man, and it can be collected in large numbers. Since the rat is susceptible to histoplasmosis under natural conditions, the procedure of making cultures from spleens and livers of a sufficient number of rats trapped in a given area may be expected to demonstrate the presence of histoplasmosis if it occurs in that area. Since proved human histoplasmosis is so infrequent, isolation of *Histoplasma* from rats or other animals (*4*, *7*) offers a direct method for obtaining information about the geographic distribution of this important disease.

Sporadic human cases of proved histoplasmosis have occurred in many parts of the world and it is reasonable to assume, therefore, in the absence of contrary evidence, that the disease has a very wide geographic distribution. Since histoplasmosis has been observed in many parts of the world, it is erroneous to assume that it has a limited "endemic" distribution in eastern central United States, unless it can be shown that the disease is actually unique to that area. The demonstration of histoplasmosis in the rat in an area such as Georgia, where autochthonous human histoplasmosis has not yet been reported, conclusively proves the presence in the area of *H. capsulatum*. It suggests that man is exposed to the pathogen and that human histoplasmosis may occur in Georgia although not previously recognized.

In an attempt to learn whether rats were infected with *Histoplasma* in geographic areas outside Loudoun County, Virginia (*4*), cultures were made from rats being collected for quite another purpose, i. e., typhus studies (*5*) in four counties in southern Georgia. During July

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and August, 1948, cultures were made from 288 rats collected in Decatur, Grady, Thomas, and Brooks Counties, Georgia. These counties lie in southwestern Georgia adjacent to the Georgia-Florida State line. The rats were taken from premises within regular trapping stations as well as from other premises selected to give a representative coverage of the four counties. They were identified as to place of capture by the carefully prepared grid pattern established for sampling the area.

Although the 288 rats of this first series came from premises distributed over the study area, they did not represent a random sample. They were selected from the total of rats examined for typhus and studied during July and August on the basis of size of animals, the availability of time of the local personnel required to make this additional study, and an approximately equal distribution of cultures among the four counties. Cultures for *Histoplasma* were made only from rats from which blood samples were obtained in sufficient volume to permit serologic examinations for both typhus and histoplasmosis.

Cultures were made from a second series of 474 animals between December 1 and December 15, 1948. This series included all animals trapped for typhus studies during the period with the exception of 25 animals which either had been bled and eviscerated before they were brought to the laboratory or were inadvertently missed. Besides the 474 animals, cultures were made from 90 animals of various species which were dead upon arrival at the laboratory and so were not

Animals examined for histoplasmosis (by culture)

Species	Brooks County		Thomas County		Grady County		Decatur County		Total	
	Number examined	Positive	Number examined	Positive	Number examined	Positive	Number examined	Positive	Number examined	Positive
First series:										
Rattus norvegicus (brown rat)	2	0	39	2	40	1	62	2	143	5
Rattus rattus (roof rat)	69	1	33	0	33	0	10	1	145	2
Second series:										
R. norvegicus	3	0	66	0	53	0	46	0	168	0
R. rattus	81	2	86	0	40	0	74	0	281	2
Spilogale putorius (spotted skunk)	0	0	1	1	0	0	2	2	3	3
Other species [1]	3	0	0	0	13	0	6	0	22	0
Third series:										
R. norvegicus	25	0	72	2	51	0	0	0	148	2
R. rattus	153	0	150	0	126	0	0	0	429	0
S. putorius	1	1	1	1	1	0	0	0	3	2
Other species [2]	0	0	4	0	45	0	0	0	49	0
Totals	337	4	452	6	402	1	200	5	1,391	16

[1] Includes 14 house mice (*Mus musculus*); two each of cotton rat (*Sigmodon hispidus*) and eastern cottontail (*Sylvilagus floridanus*); and one each of gray fox (*Urocyon cinereoargenteus floridanus*), cat squirrel (*Sciurus caroliniensis*), fox squirrel (*S. niger niger*), and domestic cat (*Felis domestica*).

[2] Includes 39 cotton rats, 6 cotton mice (*Peromyscus gossypinus*) and one each of opposum (*Didelphis virginiana pigra*), raccoon (*Procyon lotor elucus*), fox squirrel and gray fox.

suitable for typhus studies. Those included 66 rats, 20 house mice, 2 cotton rats, 1 gray fox and 1 weasel. *Histoplasma* was not isolated from any of the latter animals and they will not be discussed further.

A third series was studied from February 10, 1949 to March 3, 1949, when cultures were made from 577 rats and 3 skunks taken for typhus studies from Grady, Thomas, and Brooks Counties. Typhus studies had been discontinued in Decatur County. Cultures were also made from 39 cotton rats, 6 cotton mice, 1 fox squirrel, 1 opposum, 1 raccoon, and 1 fox.[1]

The table shows the species of animals from which cultures were obtained, their distribution by counties, and the instances in which *Histoplasma* was isolated in culture. *R. norvegicus* and *R. rattus* were caught in greatest numbers and cultures were made from 288 rats in the first series (July–August 1948), 449 in the second (December 1948), and 577 in the third (February–March 1949). The numbers of other species were too small to be of great interest with the exception of the spotted skunk which will be referred to later.

Procedures

The animals were brought to the laboratory alive in cloth bags, and location of capture, species, sex, length and weight were recorded. The animal was anesthetized; the abdominal and thoracic cavities were opened, and the animal was bled by thrusting a sterile glass tube with capillary tip into the heart. Pieces of tissue were then taken with sterile instruments from spleen and liver for culture. A piece of spleen 0.1–1.0 cc. in size was streaked over the surface of a slant of modified Sabouraud's agar [2] and, in most cases, left near the top of the slant. The size of the piece of tissue varied with the size of the organ and from series to series. In general, larger pieces were used in cultures from the third series. A similar culture was made from the liver. These cultures were incubated at room temperature until a 30° incubator was available, in most cases after a lapse of 2 or 3 weeks.

Mycologic Studies

H. capsulatum was isolated from five specimens of *R. norvegicus* and two of *R. rattus* in the first series, from two specimens of *R. rattus* in the second series, and from two of *R. norvegicus* in the third, and from three specimens of the spotted skunk (*Spilogale putorius*) in the second and two of this animal in the third.

Although *Histoplasma* was isolated from 2.4 percent of the rats cultured in the first series, this incidence was not found in the two subsequent series. This discrepancy has not been satisfactorily

[1] Made available through the courtesy of E. V. Komarek.

[2] Difco neopeptone 1 percent, dextrose 2 percent, agar 2 percent.

explained. It seemed possible that the selection of rats in the first series on the basis of size, as explained above, might have been a factor. When the rats of this series were segregated as to species and then divided into quartiles on the basis of increasing body length, the five infected Norway rats fell into the fourth quartile. However, in the case of the roof rat, one of the two infected rats fell into the fourth group and one fell into the second group. Both the infected roof rats in the second series fell into the second quartile of that series. One of the two infected Norway rats of the third series fell between the second and third, and the other between the third and fourth quartiles of their series. It is apparent, therefore, that histoplasmosis does not occur exclusively in old, large rats. It is possible that there is a seasonal variation in the incidence of histoplasmosis in the rat, but there is no clear indication of this in the data obtained to date in Virginia and Georgia.

Although the number of skunks is too small to draw conclusions about frequency of infection, it is interesting to note that *H. capsulatum* was isolated in culture from all of three skunks (*S. putorius*) trapped in three widely separated locations in the study area in December and from two of three specimens of this animal trapped on other farm premises in February. This appears to indicate a high incidence of histoplasmosis in the skunk. It should be recalled that several other species of small mammals have been unsuccessfully examined for histoplasmosis (*4*).

Macroconidia [3] representing the saprophytic growth phase of *Histoplasma capsulatum* have been demonstrated recently in soil (*1*). It might be assumed that the ground-dwelling animals are exposed to a more or less equal extent to any fungi growing in the surface layers of soil. However, the extent of the growth of *Histoplasma* in soil and the factors influencing the relative degree of exposure among ground-dwelling animals are not known.

All strains of *H. capsulatum* isolated from rats and skunks in Georgia were alike in appearance and virulence. The gross colony characteristics of the 16 Georgia strains are typical of the species. Growth rate is slow, the color is white in the young colony and becomes yellowish to brown in old cultures. These strains differed from most others we have studied in the paucity of macroconidia and the abundance of microconidia. Typical macroconidia with finger-like appendages covering the walls have been found in all Georgia strains (fig. 1), but they are found infrequently or not at all in many subcultures. However, there are enormous numbers of small conidia in all strains (fig. 2). These are typical of those seen, usually in much fewer numbers, in other strains of *H. capsulatum*. They are nearly sessile to

[3] For a discussion of terminology of *Histoplasma* spores see Emmons (*1*).

stipitate, 2–4μ in diameter, with walls which are smooth or slightly roughened to spiny. The presence of large numbers of these microconidia and the near lack of macroconidia in these Georgia strains

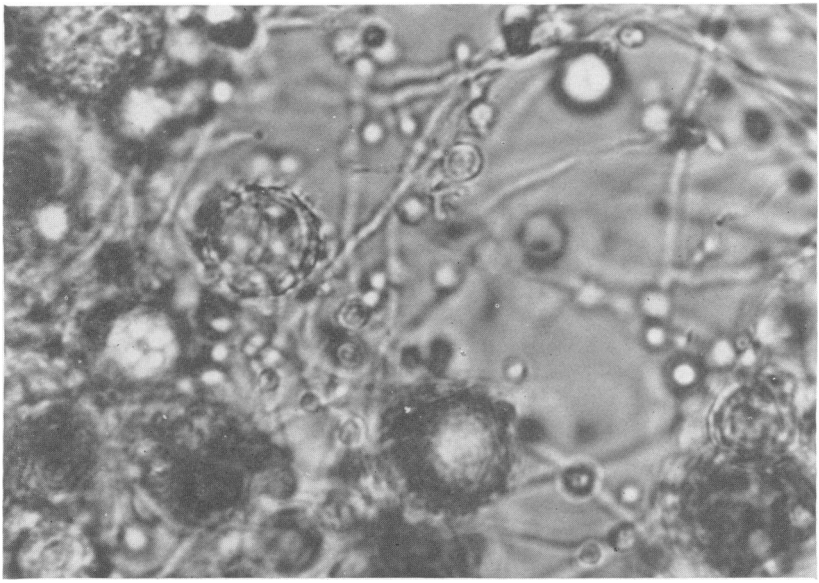

Figure 1. Macroconidia and microconidia of a strain of *H. capsulatum* isolated from a Georgia rat (×1000).

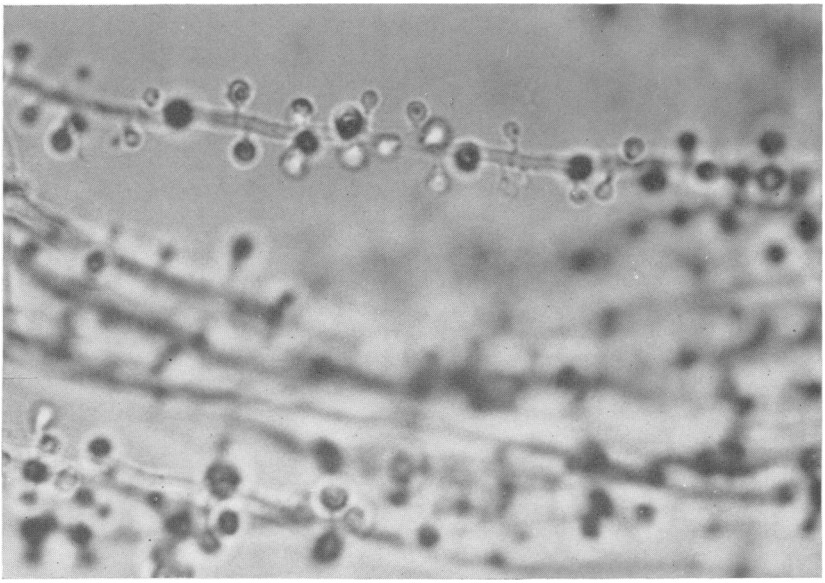

Figure 2. Abundant production of microconidia typical of Georgia rat strains of *H. capsulatum* (×1000).

makes their resemblance to cultures of *Blastomyces dermatitidis* and *Haplosporangium parvum* even greater than in most strains of *H. capsulatum*.

The production of such large numbers of small conidia is a striking feature, and it is particularly interesting that it should characterize all the Georgia strains so far seen. It cannot be considered a characteristic peculiar to strains of rodent origin, because the strains isolated from rats in Virginia bore typical macroconidia and were like strains of human origin in all observed respects. The senior author has observed numerous small conidia in one strain isolated from a cat and in a few strains from human histoplasmosis, and this characteristic sometimes appears as a transitory character in other strains carried in culture in the laboratory.

These strains from animals collected in Georgia appear to be like the strain of *H. capsulatum* isolated from the first case of South American histoplasmosis. Negroni (*6*) described this strain as characterized by the production of large numbers of small spores which he called conidia, and less numerous large spores which he called hypnospores. He noted that both types of spores might be either smooth or rough-walled. The surface markings in the small spores were described as pits, and in the large spores the familiar finger-like appendages were described and illustrated although considerable variation in the size and shape of these surface markings was noted.

Tissues were saved from only a few animals in these studies and the histopathology in these naturally infected rats from Georgia is not known. However, the 16 Georgia strains of *Histoplasma* have been tested for pathogenicity. Mice inoculated intraperitoneally with suspensions of conidia died within 3–5 weeks. The rapidly fatal development of experimental histoplasmosis does not necessarily indicate greater virulence of the Georgia strains. It seems to be related, rather, to the very numerous microconidia produced by these strains and the relative ease with which an inoculum containing a large number of infective elements can be prepared. Experimentally infected mice showed typical lesions of fatal histoplasmosis with enormously enlarged spleens and livers and peritonitis with accumulation of straw-colored fluid in the peritoneal cavity (fig. 3). Impression smears of liver and spleen reveal large numbers of *Histoplasma* cells which are typical of those previously seen and described in man, in the dog, and in experimentally infected animals (fig. 4). All strains were re-isolated from experimentally infected mice in the yeast-like form on blood agar.

Serologic Studies

A portion of the serum from each of the 286 rats in the first series was examined for complement-fixing antibodies by S. B. Salvin (*8*). The results were not informative. No fixation in the presence of

Histoplasma yeast phase antigen was observed in any of the seven rats in this series from which *Histoplasma* was isolated. No sera from rats in the second and third series were tested for fungus antibodies.

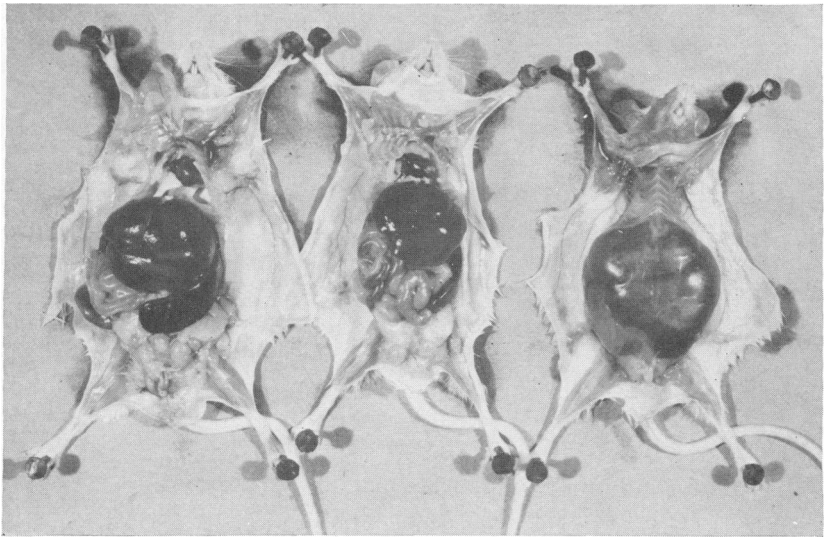

Figure 3. Mice infected with Georgia strains of *H. capsulatum.*

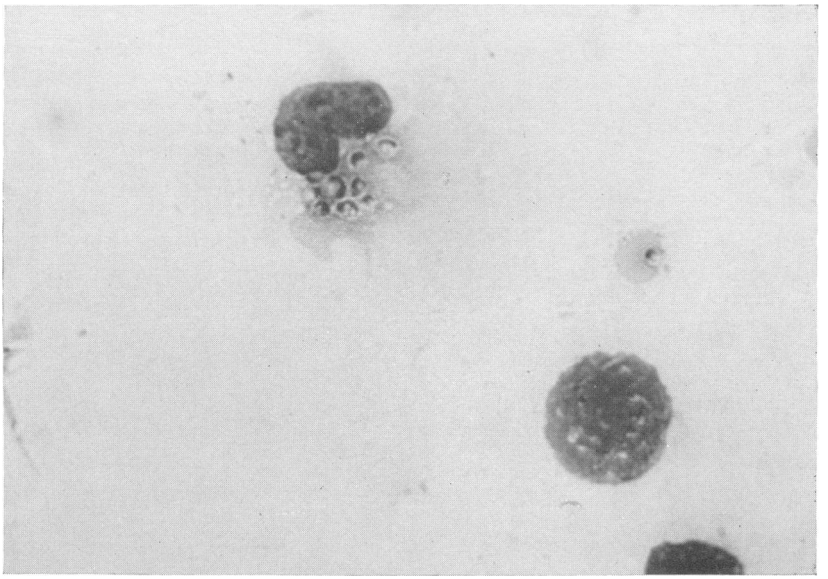

Figure 4. *H. capsulatum* in an impression smear of spleen from a mouse infected with a Georgia strain (×1000).

Discussion

This report adds two new animal species (*Rattus rattus* and *Spilogale putorius*) to the list of natural hosts of *Histoplasma* and extends the geographical limits of the known occurrence of histoplasmosis in the brown rat. No significant relationship between human and animal histoplasmosis has yet been observed. We have not been able to find any record of proved human histoplasmosis known to have been acquired in Georgia, but the isolation of *H. capsulatum* from animals in this area proves conclusively that the pathogen is present as a potential hazard.

The implications of the demonstration of *Histoplasma* in an area usually considered far beyond the limits of the area of high incidence of histoplasmin sensitivity and nontuberculous calcification will be discussed in a subsequent report.

Summary

H. capsulatum was isolated from seven brown rats (*R. norvegicus*), four roof rats (*R. rattus*) and five spotted skunks (*S. putorius*) in southwestern Georgia.

These 16 strains resemble each other but differ from most strains of human, canine, and rodent origin in the paucity of macroconidia and great abundance of microconidia. They are pathogenic for mice, and all have been re-isolated from experimentally infected mice on blood agar in the typical yeast-like form of growth.

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INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED OCTOBER 22, 1949

The reported incidence of poliomyelitis declined only slightly during the current week—from 1,207 cases last week to 1,148, a decrease of about 5 percent, as compared with nearly 24 percent last week. Figures for the corresponding week last year and the 5-year (1944–48) median are 1,078 and 722, respectively. Increases totaling 52 cases were recorded in the West North Central, West South Central, and Pacific areas. Of 25 States reporting a combined increase of 158 cases, 5 (Wisconsin, Minnesota, Missouri, Texas, and California) showed increases of from 17 to 24 cases each. States reporting currently more than 18 cases and showing increases are as follows (last week's figures in parentheses): Massachusetts 51 (50), Pennsylvania 36 (33), Ohio 52 (47), Wisconsin 43 (26), Minnesota 40 (23), Missouri 50 (27), Nebraska 24 (21), Arkansas 22 (9), Oklahoma 31 (29), Texas 57 (36), Utah 22 (19), California 98 (74).

The total for the year to date is 37,087 cases, as compared with 22,588 for the same period last year and a 5-year median of 16,856. Percentages, by geographic divisions, of the 36,171 cases reported since March 19 (same period last year 22,238) are as follows (corresponding percentages last year in parentheses): New England 8.5 (1.6), Middle Atlantic 18.9 (11.4), East North Central 24.1 (15.7), West North Central 16.2 (17.3), South Atlantic 4.4 (18.4), East South Central 4.3 (3.8), West South Central 11.9 (9.7), Mountain 4.8 (2.7), Pacific 6.9 (19.4).

During the week, 1 case of anthrax was reported in Pennsylvania, and 1 case of leprosy in California. Of the other diseases reported in the table, current figures are slightly above the corresponding medians for meningococcal meningitis, infectious encephalitis, Rocky Mountain spotted fever, and tularemia.

A total of 8,887 deaths was recorded during the week in 94 large cities in the United States, as compared with 8,750 last week, 8,974 and 8,721, respectively, for the corresponding weeks of 1948 and 1947, and a 3-year (1946–48) median of 8,785. The total for the year to date is 384,513, as compared with 385,943 for the corresponding period last year. Infant deaths totaled 644, last week 668, corersponding week last year 701, 3-year median 704. The cumulative figure is 27,495, same period last year 28,066.

Telegraphic case reports from State health officers for the week ended Oct. 22, 1949

(Leaders indicate that no cases were reported)

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [a]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine				23		10	10		6				10	
New Hampshire				1			1		2					
Vermont				2			5		2				1	
Massachusetts	5	1		35	2		51		32		1	1	54	
Rhode Island				1		2	5		1				7	
Connecticut	1			13	1	28	17		3				40	
MIDDLE ATLANTIC														
New York	6		[c]	75	4	162	138		[d] 48			2	166	17
New Jersey			2	27	1	65	58		12			1	129	1
Pennsylvania	8			61	2	29	36		19			4	155	
EAST NORTH CENTRAL														
Ohio	5			15	8	53	52		86			1	73	6
Indiana	4	1		8	1	2	22		14				22	7
Illinois	1	1		14	4	69	63		23			2	92	1
Michigan [b]	5			50	5	39	[1] 67		34				87	
Wisconsin			5	59	1	3	43		16				64	1
WEST NORTH CENTRAL														
Minnesota	1	2		5	2	3	40		16			1	1	
Iowa				10	1		15		10				4	5
Missouri	2			2	1	11	50		11		4	6	9	
North Dakota			1	3	1		2		1					
South Dakota				1		1	7		[d] 2				8	
Nebraska	1		4	3			24		4				4	
Kansas	3			1	2	13	23		12			2	2	
SOUTH ATLANTIC														
Delaware	1			1			4		5				33	
Maryland [b]	2			6		7	14	1	7			1	40	
District of Columbia				3		8	3		3					
Virginia	7	1	160	9		35	13	1	14			9	19	2
West Virginia	10			7	2	7	7		15			1	38	
North Carolina	28	1		20			6	1	87			2	24	
South Carolina	14		8		12	7			4			9	7	2
Georgia	17		13			10	[1] 10	1	14			1	3	7
Florida	7			4		15	3		7		1		4	2

EAST SOUTH CENTRAL														
Kentucky	16				4	1	18		73			1	40	16
Tennessee	7		12	7	4	43	15		56			2	8	
Alabama	6		35	2	3	16	1	1	20				9	8
Mississippi [a]	9		4	1		22	9		7		1	3	11	
WEST SOUTH CENTRAL														
Arkansas	7		42	1	2	22	[1] 22		1		5	4	9	
Louisiana	5			1		1	5		5		1		4	
Oklahoma	2	1	7	1	1	7	31		6		1		1	3
Texas	34	2	783	17	5	215	57		15		1	7	52	16
MOUNTAIN														
Montana			4	32	1		5		3					
Idaho			25	6		9	18		[d] 25				7	
Wyoming	1			4			2							
Colorado		2	26	3	1	17	13		2				6	
New Mexico			1	12		11	9	1	7			2	3	
Arizona			110	46		8	1		2			4	10	
Utah [a]	2		2		1	2	22				1		7	
Nevada				1	1				1					
PACIFIC														
Washington	1		21	28		1	18		35				6	
Oregon	4		11	15	1	13	15		7				7	
California	7	3	9	38	5	23	98		[d] 52			9	93	1
Total	229	15	1,285	674	70	990	1,148	6	827		16	75	1,369	
Median, 1944-48	385	14	1,688	922	62		722	2	1,386	3	10	100	1,539	
Year to date, 42 weeks	5,965	647	85,224	592,324	2,775	63,755	[1] 37,087	547	63,053	44	951	3,168	50,780	
Median, 1944-48	9,720	529	200,048	558,200	5,002		16,856	506	94,441	295	761	3,464	80,438	
Seasonal low week ends	(27th) July 9		(30th) July 30	(35th) Sept. 3	(37th) Sept. 17		(11th) Mar. 19		(32d) Aug. 13	(35th) Sept. 3		(11th) Mar. 19	(39th) Oct. 1	
Since seasonal low week	2,197		9,357	3,806	259		[1] 36,171		4,793	3		2,708	4,178	
Median, 1944-45 to 1948-49 [b]	3,885		10,219	4,647	336		16,593		8,146	21		2,989	4,563	

[a] Period ended earlier than Saturday.
[b] The median of the 5 preceding corresponding periods (1944-45 to 1948-49).
[c] New York City only.
[d] Includes cases reported as streptococcal infection and septic sore throat.
[e] Including paratyphoid fever; currently reported separately as follows: New Jersey 1, Virginia 1, Texas 1, California 8. Cases reported as salmonella infection, not included in the table, were as follows: Massachusetts 7, New York 2.
[1] Poliomyelitis—Deductions: Michigan, weeks ended September 3, and 24, 1 case each; Georgia, week ended October 1, 1 case; Arkansas, week ended October 1, 1 case.

Anthrax: Pennsylvania, 1 case.
Leprosy: California, 1 case.
Alaska: Measles 54, pneumonia 1, scarlet fever 2.
Hawaii Territory: Poliomyelitis 1, whooping cough 1.

FOREIGN REPORTS

CANADA

Provinces—Notifiable diseases—Week ended October 1, 1949.—During the week ended October 1, 1949, cases of certain notifiable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox	1		17	3	57	56	5	14	31	46	230
Diphtheria			1		3	3	1				8
Dysentery, bacillary					12	4					16
Encephalitis, infectious							3				3
German measles			1			6			23	12	42
Influenza			7			14	3				24
Measles	1		34		59	42	11	35	33	120	335
Meningitis, meningococcal					1	1					2
Mumps			8		31	79	3	6	2	55	184
Poliomyelitis	5		4	1	12	30	12	3	6	6	79
Scarlet fever	2		1	1	26	27	6	1	10	4	78
Tuberculosis (all forms)	13			5	159	23	21	6		57	284
Typhoid and paratyphoid fever	1				16	6			3	2	28
Undulant fever									1		1
Venereal diseases:											
Gonorrhea	17	3	8	16	83	87	30	17	44	83	388
Syphilis	5		19	5	56	23	13	10	4	38	173
Other forms										1	1
Whooping cough	1			1	98	49	5	12	2	5	173

CUBA

Habana—Notifiable diseases—4 weeks ended August 27, 1949.—During the 4 weeks ended August 27, 1949, certain notifiable diseases were reported in Habana, Cuba, as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Diphtheria	9		Tuberculosis	7	2
Leptospirosis	1		Typhoid fever	27	1
Measles	1	1	Undulant fever	1	

Provinces—Notifiable diseases—4 weeks ended August 27, 1949.—During the 4 weeks ended August 27, 1949, cases of certain notifiable diseases were reported in the Provinces of Cuba as follows:

Disease	Pinar del Rio	Habana[1]	Matanzas	Santa Clara	Camaguey	Oriente	Total
Cancer	4	8	12	23	1	15	63
Chickenpox						1	1
Diphtheria		10	6	1	1	3	21
Leprosy		7				1	8
Malaria	3	2	2	1	7	19	34
Measles		3	7		3		13
Poliomyelitis	1	1			1	1	4
Tetanus					1		1
Tuberculosis	6	18	13	47	2	18	104
Typhoid fever	14	46	9	15	16	43	143
Undulant fever		1				3	4
Whooping Cough					1		1

[1] Includes the city of Habana.

JAMAICA

Notifiable diseases—5 weeks ended October 1, 1949.—For the 5 weeks ended October 1, 1949, cases of certain notifiable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Cerebrospinal meningitis		1	Poliomyelitis		1
Chickenpox	7	15	Puerperal sepsis		1
Diphtheria	2	6	Scarlet fever		1
Dysentery, unspecified	1	1	Tuberculosis (pulmonary)	38	63
Erysipelas	1	2	Typhoid fever	2	51
Leprosy		3	Typhus fever (murine)	1	

NORWAY

Notifiable diseases—July 1949.—During the month of July 1949, cases of certain notifiable diseases were reported in Norway as follows:

Disease	Cases	Disease	Cases
Cerebrospinal meningitis	7	Paratyphoid fever	3
Diphtheria	24	Pneumonia (all forms)	1,301
Erysipelas	316	Poliomyelitis	7
Gastroenteritis	3,625	Rheumatic fever	82
Gonorrhea	237	Scabies	823
Hepatitis, epidemic	80	Scarlet fever	249
Impetigo contagiosa	1,539	Syphilis	49
Influenza	975	Tetanus	1
Laryngitis	5,430	Tuberculosis (all forms)	295
Malaria	1	Typhoid fever	1
Measles	1,337	Whooping cough	3,529
Mumps	247		

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

Note.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Plague

Peru.—During the period September 1–30, 1949, plague was reported in Peru as follows: At Barraza Farm, Trujillo Province, Libertad Department, 1 case; at St. Nicholas Farm, Chancay Province, Lima Department, 1 case.

Union of South Africa.—Plague has been reported in Union of South Africa as follows: In Cape Province—week ended October 1, 1949, 1 case at Witwater Farm, Kuruman District, week ended October 8, 1 death at Clapin Farm, Kuruman District, 1 case in Olifantshoek Municipal Location; in Orange Free State—week ended October 1, 1 death (suspected plague) at Aloe Farm, Heilbron District, week ended October 8, 1 death (pneumonic plague, suspected) at Twyfelfontein Farm, Ladybrand District.

Smallpox

French West Africa—Ivory Coast.—For the period September 21–30, 1949, 41 cases of smallpox, with 15 deaths, were reported in Ivory Coast, French West Africa.

Mexico.—During the week ended September 17, 1949, 34 cases of smallpox were reported in Mexico, of which 29 cases occurred in Otzoloapan, Mexico State.

Union of South Africa—Transvaal.—Smallpox has been reported in Transvaal, Union of South Africa, as follows: May 1–31, 1949, 94 cases; June 1–30, 166 cases, 20 deaths; July 1–31, 145 cases, 6 deaths.

Typhus Fever

Portugal.—During the month of June 1949, 1 death from typhus fever was reported in Aveiro District, Portugal, and during the month of July, 1 death from this disease was reported in Braga District.

Spain—Madrid.—During the week ended September 3, 1949, 1 case of typhus fever was reported in the city of Madrid, Spain.

Union of South Africa.—During the period May 1–July 31, 1949, 42 cases of typhus fever were reported in Union of South Africa, distributed as follows: May 1–31, Cape Province 12 cases, Natal 4 cases, Transvaal 1 case; June 1–30, Cape Province 8 cases, Natal 1 case; July 1–31, Cape Province 7 cases, Natal 8 cases, Transvaal 1 case.

DEATHS DURING WEEK ENDED OCT. 22, 1949

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Oct. 22, 1949	Corresponding week, 1948
Data for 94 large cities of the United States:		
Total deaths	8,887	8,974
Median for 3 prior years	8,785	
Total deaths, first 42 weeks of year	384,513	385,943
Deaths under 1 year of age	644	701
Median for 3 prior years	704	
Deaths under 1 year of age, first 42 weeks of year	27,495	28,066
Data from industrial insurance companies:		
Policies in force	70,102,189	70,838,716
Number of death claims	12,171	12,861
Death claims per 1,000 policies in force, annual rate	9.1	9.5
Death claims per 1,000 policies, first 42 weeks of year, annual rate	9.1	9.3

QUARANTINE PROVISIONS OF IRELAND

The Government of Ireland has pointed out that vaccination against smallpox is not required of persons entering Ireland. However, that country has a provision that persons coming from known smallpox areas may be required to submit to quarantine or other restrictions if they do not possess vaccination certificates.