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*Bacteriological and Practical
Aspects of*

**PAPER
CONTAINERS
FOR MILK**

By M. J. Prucha
and P. H. Tracy

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Bulletin 495

UNIVERSITY OF ILLINOIS
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Bacteriological and Practical Aspects of Paper Containers for Milk

By M. J. PRUCHA and P. H. TRACY*

SINCE the beginning of the market-milk industry health officials have recognized the need of a sanitary container for distributing milk to the consumer. The first step in the development of such a container was the glass milk bottle, which was introduced in 1878. Altho the first paper milk container was invented in 1906, the glass bottle remained in almost universal use until 1929, when a paper container was introduced by a large milk dealer in New York City. Since this time, with the marked trend from retail to store sales of milk, for which a single-service container is especially well adapted, the paper container has come to be widely used.

As the paper bottle began to be used more extensively for the distribution of fluid milk, its merits were brought up for more specific consideration. Altho the single-service feature of the paper container has its obvious advantages, questions were raised as to its bacterial condition and whether, from a physical standpoint, it could give as satisfactory service as the glass bottle. To help answer some of these questions, the experiments reported in this bulletin were made in the Department of Dairy Husbandry at the University of Illinois.

The experiments dealt with various bacteriological problems connected with making the paper, fabricating the paper containers and handling them at the milk plant. The study also included problems pertaining to the physical condition of the paper containers, their adaptability to milk distribution, and consumer reaction to them. Economic problems connected with the use of the containers were not studied.

TYPES OF CONTAINERS STUDIED

Altho numerous types and shapes of paper milk containers have been put on the market, this study was confined to the three most common types used in this country at the time of the study (Fig. 1). These three types were made from paperboard manufactured at widely separate mills, and the containers were fabricated at several different factories.

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Fig. 1.—The three types of paper milk containers shown here are representative of those in use when this study was made: (A) Pure-Pak, (B) Seal-right, and (C) Canco.

Pure-Pak container. This container is partly prefabricated at the carton factories where it is designed, cut out, printed, sealed along its long edge, and finally packed in cartons for shipment to milk plants. At the milk plants, the container is paraffined and then filled with milk. A special machine is needed for this purpose (Fig. 2). The machine has five compartments or units. The containers enter at one end, where they are shaped and the bottoms are sealed with an adhesive. The containers next enter the paraffining unit. Here they remain 28.12 seconds: 3.75 seconds in the hot air above the paraffin, 11.25 seconds submerged in the hot paraffin, and 13.12 seconds in the hot air above while the paraffin is being drained out of the container. At the end of each day's run the paraffin is drained from the well. When any paraffin is added, it is first melted and strained.

From the paraffining unit the container goes to the cooling unit, where the paraffin is hardened. Next it enters the filling unit. When the container is filled it passes into the sealing unit. Here the steam defoamer flattens down any foam on the top of the milk, and the

container is sealed by means of an electrically heated knife. The knife melts the paraffin for an instant along the edge to be sealed and quickly presses the edges of the opening together, the paraffin acting as a seal. The opening is further fastened by means of a wire staple clamp. The filled and sealed container is dated and then leaves the machine.

In this study the regular large-size commercial machine for paraffining and filling the containers was used. It was loaned to the University for this investigation by the Ex-Cell-O Corporation of Detroit. The company also donated all the containers necessary for the study.

Canco container. This container (Fig. 1, C) is completely fabricated and paraffined at the carton factory, packed in sealed cartons, and shipped to milk plants. Here the containers are fed to a specially made filling machine (Fig. 3) which automatically opens the container, fills it with milk, and seals and dates it. The machine used in this study was the regular commercial model made by the American Can Company of New York City and loaned to the University. The company also donated all the containers necessary for the study.

Sealright container. This container is completely fabricated and paraffined at the carton factory and shipped to milk plants as needed. The container (Fig. 1, B) differs from the Pure-Pak and the Canco container in that it is conical in shape and has a circular opening similar to that of the glass milk bottle. To fill the containers the regular glass-bottle filler equipped with special capping attachments was used. The

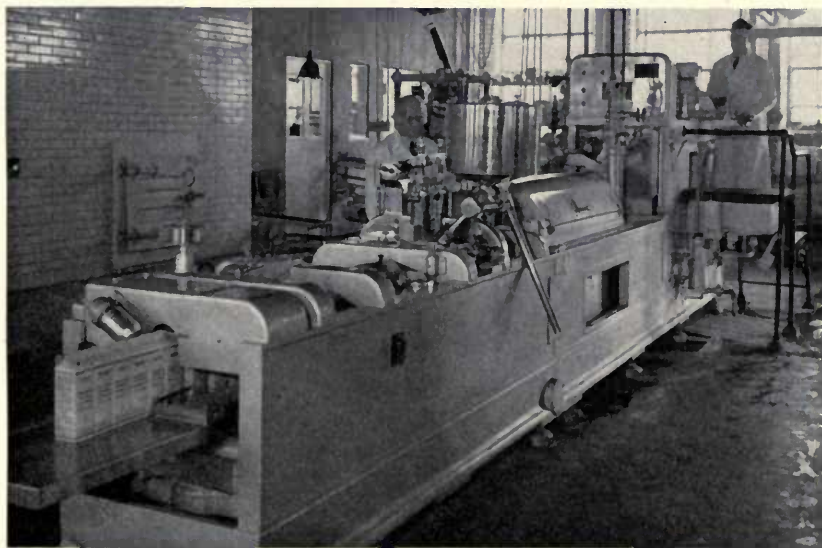


Fig. 2.—Machine in which Pure-Pak containers were paraffined and filled.

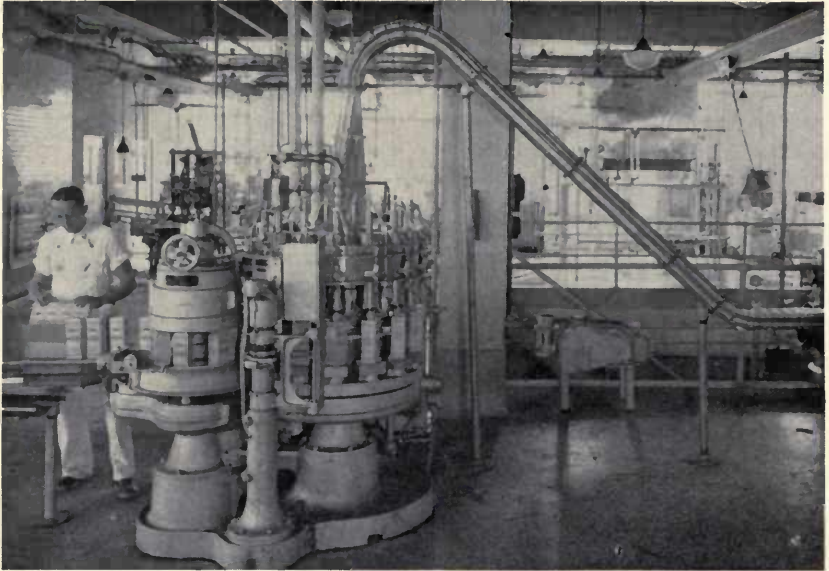


Fig. 3.—Canco containers were filled in this machine, having been fabricated and paraffined at the factory.

Sealright Company of Fulton, New York, makers of these containers, loaned the attachments and donated all the containers needed for the study.

PART I

SANITARY ASPECTS OF PAPER MILK CONTAINERS

Bacteriological Survey of Papermaking

In order to point out the bacteriological problems involved in making paper milk containers, a brief study of pulp and paper manufacture was made. The study was confined to an inspection of seven paper mills and to a bacteriological examination of the processes of two paper mills.

The paper used in the manufacture of milk containers is made from wood pulp, mostly from red spruce or yellow pine (Fig. 4). Usually the paper mills are located adjacent to an abundant water and wood supply, but some mills get the logs from distant regions when there are convenient transportation facilities.

Pulp making and papermaking are two separate processes. Some

mills make only pulp, some only paper, and others make both pulp and paper. Normally a great deal of the pulp is imported from Europe and Canada.

In making pulp, the logs are first stripped of bark. They are then conveyed from the storage pile into a large revolving drum, the washing machine, where they are thoroly cleaned. The cleaned logs are then



Fig. 4.—Paper for milk containers is made only from logs.

carried on a conveyer to the chipping machine. From here the small chips of wood are conveyed into the cookers, which are large steel cylinders lined with brick. In these cylinders the chips of wood are mixed with sulfur-lime solution or with an alkaline solution and the mixture is cooked by indirect steam at about 240° F. for several hours.

The cooking digests certain materials in the wood and liberates and softens the cellulose fiber which becomes the pulp. The pulp is diluted with copious amounts of water and is passed thru a series of operations in which it is cleaned and freed of undigested particles and dirt.

The next step in the operation is the bleaching process, which is carried out in large white tile-lined tanks (Fig. 5). Calcium hypochlorite is used as the bleaching agent.

After the pulp is bleached it is conveyed from the tanks into tile-

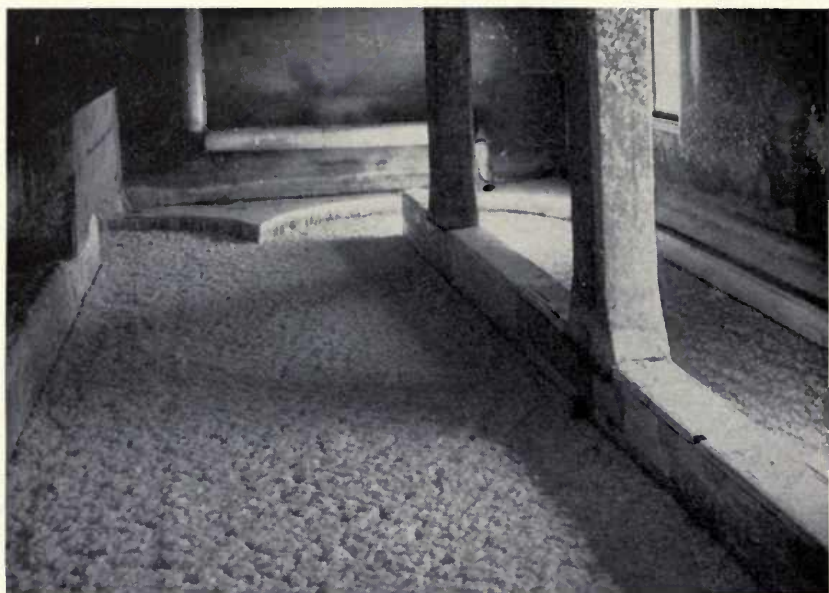


Fig. 5.—Pulp used for making paper milk containers is bleached in white tile-lined vats with a strong solution of calcium hypochlorite. This process makes the pulp sterile.

lined drainers, where excess chlorin and bleaching residue are removed by washing. This completes the pulp-mill operations.

At this point the pulp may be handled in one of three ways. If it is to be shipped to distant paper mills, it is pressed into thick sheets and these are dried. If the paper mill is connected with the pulp mill, the pulp may either be made directly into paper or it may be passed thru presses to free it of excess water and then stored without being dried. When paper is made from the dry sheets of pulp or from the pulp that has been stored moist, it is first fed to beaters, where it is mixed with water and agitated enough to separate the individual fibers. Just before the mixture of pulp and water enters the paper mill, the size is added. This consists of an emulsion of partially saponified rosin and papermakers' alum: $\text{Al}_2 (\text{SO}_4) 3.18 \text{H}_2\text{O}$. After the size is added, the mixture is passed thru a conical hydrating device called the Jordan engine and then over screens to the paper mill. Here it is further diluted with water to about .5 percent or less of pulp. In this dilution the pulp passes into a vat from which it is picked up by a revolving cylinder covered with fine-mesh wire cloth or by other means. A wool felt cloth picks a layer of pulp off the wire cloth. Several such layers from successive cylinders are combined to form a single layer which, when dried, becomes the paperboard.

In the process of drying, the layer of pulp passes over hot drier rolls (Fig. 6). The number of these driers varies from 30 to as many as 150 in different paper mills. They are heated by steam, some of them reaching a temperature of about 240° F. at the surface. By the time the pulp passes over the last roll, it is in the form of paperboard and is perfectly dry. The water has been boiled off by the heat from the rolls. Finally the paper is dampened with water and passed over two or three heated calender rolls to make its surface smooth.

The paper is cut into desired shape and size, its edges are trimmed, and it is wrapped in convenient bundles to be shipped to the carton factory.

In Mill 1 the pulp was made when convenient. After it was bleached and the excess water pressed out, it was stored in large piles. At the time of this study, in February, the paper was made from bleached pulp which had been in storage in moist condition for about two weeks.

The water used in this mill was of excellent quality. Its bacterial count was 400 per milliliter before chlorination and 40 afterwards

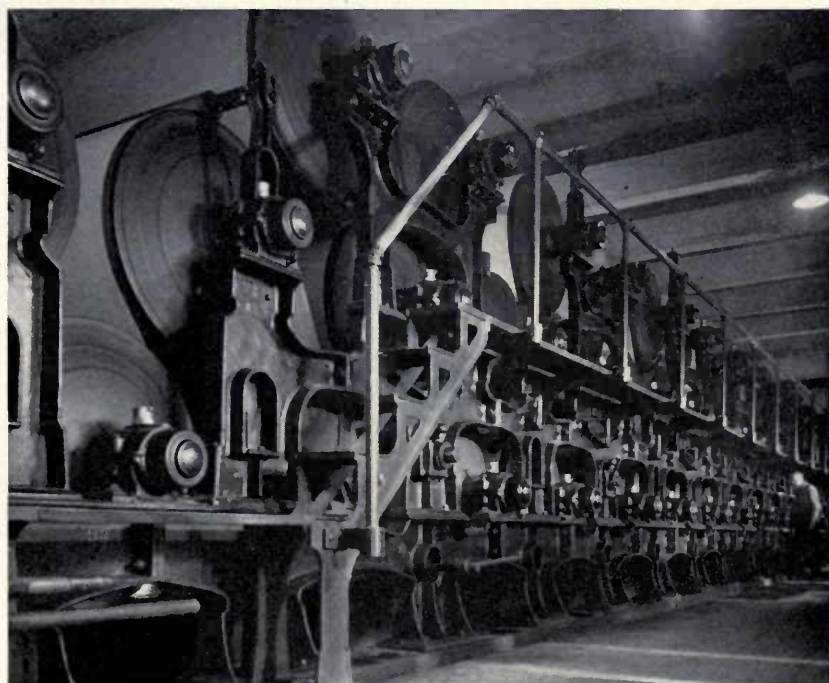


Fig. 6.—The heat in a battery of drier rolls gives the paper to be used for milk containers an incidental but important bactericidal treatment. The heat of some of these rolls runs as high as 240° F.

TABLE 1.—BACTERIAL COUNTS AND RESULTS OF TESTS FOR *E. coli* IN SAMPLES OF WATER AND PULP TAKEN AT MILL 1

Sample	Bacteria per milliliter or per gram	Test for <i>E. coli</i>
Water supply before chlorination.....	400	+
Chlorinated water entering beaters.....	50	-
Pulp entering beaters.....	280 000 ^a	-
Pulp and water mixture before size was added.....	32 000	-
Size.....	413 000	-
Alum solution (Al) ₂ (SO ₄) ₃	0	-
Mixture of pulp, water, and size.....	42 000	-
Diluted mixture of pulp, water, and size (.25 percent pulp) entering paper machine.....	1 300	-
Pulp at Cylinder 1.....	1 260	-
Pulp at Cylinder 2.....	1 020	-
Pulp at Cylinder 3.....	1 400	-
Pulp at Cylinder 4.....	910	-
Water entering top calender.....	0	-
Water entering bottom calender.....	0	-
Water from top calender trough.....	0	-
Water from bottom calender trough.....	0	-
Pulp layer entering first drier roll.....	Plates moldy	-
Finished paper.....	40 ^a	-

^aThese figures are per gram; all others are per milliliter.

(Table 1). A negative coli test was obtained from all samples except the sample of untreated water. The bacterial population of the mixture of pulp, water, and sizing was derived principally from the pulp, which had been stored in a moist condition for two weeks.

In Mill 2 the pulp was not stored but was made into paper as soon as it was cleaned and bleached. The freshly digested pulp was sterile. It became contaminated by the water during the cleaning and washing operations. The bacterial count of the water was 39,000 per milliliter (Table 2). By the time the pulp and water mixture reached the bleaching vats its bacterial count had increased to 390,000 per milliliter. The reason for this increase was not determined, but it was probably caused by the multiplication of bacteria in the mixture and by additional bacteria being picked up from the equipment.

TABLE 2.—BACTERIAL COUNTS AND RESULTS OF TESTS FOR *E. coli* IN SAMPLES OF WATER AND PULP TAKEN AT MILL 2

Sample	Bacteria per milliliter or per gram	Test for <i>E. coli</i>
Digested wood leaving the cooker as pulp.....	0	-
Pulp partly washed.....	1 600	+
Water supply.....	39 000	+
Pulp after screening and dilution with water.....	390 000	+
Bleached pulp.....	0	-
Pulp washed of bleaching residue.....	250	+
Sizing material.....	11 000 000	-
Diluted pulp entering the paper mill.....	600	+
Finished paper.....	20 ^a	-

^aThis figure is per gram; all others are per milliliter.

The bleaching process made the pulp sterile but subsequent washing to remove the residue of the bleaching agent and to dilute the pulp caused recontamination. The sizing material also added some bacteria to the mixture. The bacterial count of the pulp mixture as it entered the paper mill proper was, however, much less than that of the water used for washing (Table 2). Traces of the bleaching agent apparently remained in the pulp and continued to act on bacteria even after a thoro washing.

The bactericidal action of the drier rolls in Mill 2 was shown by comparing the *E. coli* tests for the diluted pulp entering the rolls and those for the finished paper. The water used in this mill had a high *E. coli* content and the mixture of the pulp and water gave positive tests just as the wet layer of pulp entered the drier rolls. However, the finished paper always gave negative *E. coli* tests.

There are three places in the mill where pulp and paper receive bactericidal treatment. The first place is the cooker. As the cooked wood mixture left the cooker it was entirely free from all microbial life. The second place is the bleaching vat, where very strong chlorin solution is used. The bleached pulp was sterile. The third place is at the hot steel drier rolls, which are heated indirectly with steam under pressure, some attaining a temperature of about 240° F.

The bactericidal action of the drying process was also studied by Tanner, Wheaton, and Ball.^{12*} They inoculated the wet layer of pulp or web heavily with the nonsporeforming organisms *Aerobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus peptogenes*, and also with an aerobic sporeformer just as the pulp was entering the drier rolls. Samples of the finished paper were then taken from the inoculated area for bacteriological examination. None of the nonspore-bearing organisms were recovered from the paper after it passed over the drier rolls; the sporeforming organisms survived.

Appling and Shema conducted similar tests.^{2*} They report: "When the wet web of a Fourdrinier machine was inoculated with *Escherichia coli* (a heat-resistant strain), *Serratia marcescens*, *Staphylococcus aureus*, *Aerobacter aerogenes* and with mixed suspension containing the first three nonsporeforming species in addition to *Bacillus mycoides* (a spore-former), it was found that only the sporeforming species survived the drier rolls."

Inspection of Carton Factories

Five factories where the paper milk containers were fabricated were inspected. Two factories were located at the paper mills and three were separated from the mills and received the shipments of paperboard by freight.

*Thruout this bulletin the numbers marked with an asterisk refer to the literature citations on page 472.



Fig. 7.—At the carton factory, paper for milk containers is stored in large rooms. Before the air enters the room it is washed, humidified, and heated to a uniform temperature. Note ventilator.

Upon arrival from the mill the paperboard was transferred to a large storage room (Fig. 7). This room and the rooms in which the containers were made had controlled temperature and humidity and were regularly inspected and treated to prevent the establishment of insects and vermin. There was no evidence of either insects or of the accumulation of dirt and dust in any of the rooms where the paper was handled.

In addition, the factories required medical examinations of all the employes who came in contact with the containers. Some of the companies maintained a first-aid health department and an emergency hospital.

To summarize, the inspection revealed that the carton factories which were visited were attempting to comply with all measures necessary to manufacture sanitary containers.

Bacteriological Examination of Paper

The sanitary condition of dairy utensils is judged by the number of bacteria on the surfaces, particularly on the inner surfaces that come in contact with milk. Tests were made to determine the number of bacteria on the surface of the paper by rubbing it with a sterile wet swab. The number of bacteria recovered by this method was negligible.

Another method for finding out the number of bacteria on the surface of the paper was tried. The paper was soaked in sterile water and the water was then plated. In one test 20 quart-size unparaffined Pure-Pak containers were immersed in 3 gallons of sterile water. After



Fig. 8.—The paper plaques were reduced to pulp in this machine in order that the bacteria enmeshed in the fibers could be counted.

20 minutes of soaking and after vigorous agitation ten agar plates were prepared, each receiving 1 ml. of the rinse water. Assuming that all the colonies that developed on the plates came from the surface of the paper, calculations showed that there was an average of 2.3 bacteria per square inch of paper.

Since paper differs from metal and glass in that it is much more porous, it may have bacteria enmeshed among the fibers as well as on the surface. To determine the number of bacteria among the fibers, it was necessary to reduce the paper to pulp. After various attempts a satisfactory apparatus for doing this was found (Fig. 8). It was an electrically driven stirrer such as is used at a soda fountain. The mixing cup was equipped with four long stirring blades attached to the inside bottom of the cup and rotated in a horizontal plane at high speed. The cup could easily be sterilized in autoclave.

In carrying out the tests, 2 grams of the paperboard were cut into small pieces with sterile scissors. The pieces were placed in the mixing

cup and 200 ml. of sterile water was added. After 5 minutes of mixing, the paper was completely reduced to pulp. Ten agar plates were prepared from a sample, each plate receiving 2 ml. of the mixture. The plates were incubated four days at 90° F. because some bacteria in the paper which grow very slowly do not form visible colonies until after two days of incubation.

TABLE 3.—NUMBER OF BACTERIA ENMESHED IN PAPERBOARD FROM WHICH MILK CONTAINERS WERE FABRICATED
(Samples from five mills)

Number of samples	Bacteria per gram	Number of samples	Bacteria per gram	Number of samples	Bacteria per gram	Number of samples	Bacteria per gram
5.....	0	8.....	40	3.....	150	2.....	260
4.....	4	11.....	50	5.....	160	3.....	270
2.....	6	7.....	60	1.....	170	3.....	280
17.....	10	13.....	70	4.....	180	1.....	290
3.....	13	4.....	80	4.....	190	2.....	300
2.....	16	3.....	90	2.....	200	1.....	320
1.....	19	2.....	100	4.....	210	1.....	370
4.....	20	2.....	110	2.....	220	1.....	400
5.....	25	5.....	120	3.....	230	2.....	440
1.....	27	4.....	130	2.....	240	4.....	560
1.....	28	2.....	140	4.....	250	2.....	580
9.....	30						

Bacteriological examinations were made of 170 samples of paper from five different mills (Table 3). Of these samples 2.94 percent gave no bacterial count; 40 percent gave a count of 50 or less per gram of paper; 16.46 percent gave a count of 100 or less per gram; 27.66 percent gave a count of 250 or less; and 12.94 percent gave a count of 250 to 580 per gram of paper.

Attempts have been made by milk sanitarians to adopt some bacterial standards for the paperboard from which the milk containers are to be made. For example, it is recommended in the U. S. Public Health Milk Ordinance that a bacterial plate count of 250 per gram of paper be the limit.^{15*} Paper can be made that is nearly free from bacteria or that has a bacterial count less than 250 per gram. This is evident from the results in Table 3 and from studies of other investigators.^{9, 10*}

Bacteriological Examination of Containers Before Paraffining

The Pure-Pak container is the only one of the three types of paper containers that is not completely fabricated at the factory. The bacteriological examination of the containers before paraffining was therefore confined to this container.

At the milk plant the Pure-Pak containers were passed thru the regular bottling machine, where they were shaped, the bottoms sealed with the adhesive, and the tops closed and fastened with wire staples.

The containers were selected at random from the regular stock. They were thus handled in the usual way at the carton factory, during transportation from the factory to the milk plant, and at the milk plant, except that they were not paraffined and were not filled with milk.

The method used for determining the bacteriological condition of the Pure-Pak containers was the regular Standard Method.¹ Each container was rinsed with 100 ml. of sterile water and two agar plates were prepared, each receiving 1 ml. of the rinse water. Trypton dextrose agar was used instead of the pepton agar.

Of the 128 Pure-Pak containers examined, 66 came from eleven different milk plants where paper milk containers were used and 62 came from the regular experimental supply, but all were passed thru the machine installed in the University dairy manufactures laboratory.

The average of the colony counts on duplicate plates from the 128 containers were as follows:

<i>Number of containers</i>	<i>Average of colony counts on duplicate plates</i>
25.....	0
25.....	.5
16.....	1.0
18.....	1.5
8.....	2.0
7.....	2.5
5.....	3.0
2.....	3.5
2.....	4.0
4.....	4.5
4.....	5.0
3.....	6.5
9.....	ranged from 8 to 18

It is evident that, whatever the various sources of bacteria were at the paper mill, at the carton factory, and at the milk plant, they added a very insignificant number of bacteria to the surface of the paperboard and the fabricated container.

Bacteriological Examination of the Paraffined Containers

Pure-Pak container. Two sets of these containers were examined. One set of 396 containers, representing the regular stock used in this investigation, was selected in the following manner: On sixty-six different days during the bottling of milk, 6 containers were passed thru the machine, 3 just before the bottling of milk was started, and 3 immediately after all the milk was bottled but before any cleaning of the machine was done. The containers were exposed to all the machine operations except that no milk was put in them.

The second set of Pure-Pak containers consisted of 132 and represented the regular supplies of eleven milk plants located in different parts of the country. The paperboard from which these containers

were made was manufactured in different paper mills, and the containers were prefabricated in several carton factories. The partially prefabricated containers were first shipped to the respective milk plants and thence to the dairy manufactures laboratory at the University. At the laboratory they were paraffined in the regular machine in one continuous operation and thus were exposed to all parts of the machine but no milk was put in them. The paraffin bath was held at 170° F. and the machine was geared at its regular speed (page 416).

Of the first set of 396 Pure-Pak containers 24.24 percent yielded no colonies and 75.25 percent yielded 2 colonies or less per plate after the paraffining process. Actual colony counts on the duplicate plates were as follows:

<i>Number of containers</i>	<i>Colonies on duplicate plates</i>	<i>Number of containers</i>	<i>Colonies on duplicate plates</i>
96.....	0-0	3.....	4-5
110.....	0-1	1.....	5-5
76.....	1-1	3.....	5-6
56.....	1-2	1.....	7-8
34.....	2-2	1.....	10-11
13.....	3-4	1.....	20-21
1.....	4-4		

Only 2 containers gave counts of more than 1,000 bacteria; the highest was 2,050 per container.

The results for the second set of 132 Pure-Pak containers were very similar to those of the first set: 32.58 percent yielded no colonies after paraffining and 63.63 percent yielded 2 colonies or less per plate. Actual colony counts on the duplicate plates were as follows:

<i>Number of containers</i>	<i>Colonies on duplicate plates</i>	<i>Number of containers</i>	<i>Colonies on duplicate plates</i>
43.....	0-0	4.....	2-2
42.....	0-1	3.....	2-3
25.....	1-1	1.....	3-4
11.....	1-2	1.....	9-14

Only one container gave a total count of more than 1,000 bacteria and that was 1,150.

Canco container. Since the results obtained with the Pure-Pak containers indicated that the number of bacteria in paper containers was very small, too small to be accurately determined by the Standard Method then in use, the method used for the Canco containers was modified in that no rinse water was used. The trypton-dextrose agar (25 ml.) was poured directly into each container and, after thoro agitation to bring all the inside surface of the container in contact with the agar, the containers were incubated at 90° F. To count the colonies the containers were cut open and the slab of agar was placed on the ruled glass counting plate.

The 1,155 Canco containers examined represented several different

shipments and were selected at random from the conveyer as they passed to the bottling machine. Usually from 6 to 10 containers were picked during one day's run. Of the 1,155 paraffined containers 568, or 49.17 percent, yielded no colonies and 407, or 35.32 percent, yielded 2 colonies or less per container (*not per milliliter of capacity*). The bacterial condition of the containers was as follows:

<i>Number of containers</i>	<i>Colonies in containers</i>	<i>Number of containers</i>	<i>Colonies in containers</i>
568.....	0	11.....	50 to 100
323.....	1	6.....	100 to 200
84.....	2	1.....	309
118.....	2 to 10	1.....	1 500
43.....	10 to 50		

Only one container gave a count of more than one colony per milliliter of capacity and the total count for this container was 1,500 colonies.

Sealright container. After a number of the Sealright containers were examined by the method used for the Canco containers, it was found that pouring agar directly into the containers was not successful. The slabs were difficult to remove because of the shape and construction of the containers. For this reason a modification of the method was adopted by which each container was rinsed with 10 ml. of sterile water. After the container was thoroly shaken, all the rinse water that could be recovered with a 10-ml. pipet was divided equally among three petri plates and about 15 ml. of agar was added to each plate. After incubation the colonies were counted.

The bacterial condition of the 724 paraffined Sealright containers was as follows:

<i>Number of containers</i>	<i>Range of colony counts on triplicate plates</i>	<i>Number of containers</i>	<i>Range of colony counts on triplicate plates</i>
34.....	0	120.....	101 to 1 000
261.....	1 to 10	24.....	1 001 to 5 000
284.....	11 to 100	1.....	5 300

The highest count obtained was 5,300 colonies in one container. If this container had been filled with milk it would have increased the bacterial count of the milk by 5.6 colonies per milliliter.

Comparison of methods for determining bacterial count. Since a different method was used for each of the three types of containers, it was necessary to ascertain whether the three methods gave comparable results. For this purpose lots of 50 Pure-Pak containers were examined by each of the methods. A fourth lot was examined by the method suggested by Fitzgerald,^a which consisted of rinsing each con-

^aDr. F. F. Fitzgerald was formerly manager of the research department of the American Can Company.

tainer with 20 ml. of sterile water and plating exactly 10 ml. of it, dividing the amount equally among three plates.

The first method, or the Standard Method, in which 100 ml. of rinse water was used for each container, showed 37 of the 50 containers yielding no colonies on duplicate plates each containing 1 ml. of the rinse water (Table 4). When agar was poured directly into the containers without the use of rinse water and the containers were in-

TABLE 4.—NUMBER OF BACTERIA IN CONTAINERS EXAMINED BY FOUR DIFFERENT METHODS*
(50 containers were examined by each method)

Method 1 (used for Pure-Pak tests)		Method 2 (used for Canco tests)		Method 3 (used for Sealright tests)		Method 4 (suggested by Fitzgerald)	
Number of containers	Colonies on duplicate plates	Number of containers	Colonies on agar in each container	Number of containers	Colonies on triplicate plates	Number of containers	Colonies on triplicate plates
37.....	0-0	44.....	0	30.....	0-0-0	36.....	0-0-0
9.....	0-1	4.....	1	11.....	0-0-1	8.....	0-0-1
2.....	0-2	1.....	2	2.....	0-1-1	1.....	0-1-1
1.....	1-1	1.....	110	1.....	0-1-2	2.....	0-1-2
1.....	8-17			1.....	0-0-4	1.....	0-0-4
				1.....	0-2-4	1.....	0-3-4
				1.....	2-2-3	1.....	12-19-29
				1.....	1-2-6		
				1.....	7-8-12		
				1.....	112-144-172		

*The procedure for the four methods was as follows: **Method 1**, container rinsed with 100 ml. of water; 1 ml. of recovered water put on each of two agar plates. **Method 2**, no rinse water used; agar poured directly into container and incubated. **Method 3**, container rinsed with 10 ml. of water; all recovered water divided between three agar plates. **Method 4**, container rinsed with 20 ml. of water; 10 ml. of recovered water divided between three agar plates.

cubated in the same manner as the plates, which was the method used for the Canco containers, 44 of the 50 Pure-Pak containers yielded no colonies. When only 10 ml. of water was used for rinsing and all the recovered water was plated in three plates, as was done with the Sealright containers, the triplicate plates from 30 of the 50 Pure-Pak containers yielded no colonies. When 20 ml. of water was used for rinsing and exactly 10 ml. of the water was plated in three plates, as was suggested by Fitzgerald, 36 of the 50 containers yielded no colonies on the triplicate plates.

The method used for the Canco containers, in which agar was poured directly into the containers, yielded a somewhat higher percentage of sterile containers than the other methods, in which the containers were rinsed with sterile water and agar plates were prepared from the water. In general, however, the four methods used for examining the paraffined paper containers gave very similar results. All showed that the containers were either free from bacteria or contained only a very few.

Summary of tests with paraffined containers. Of the 2,607 paraffined containers examined, 878 were sterile and only 29 gave a count of more than one bacterium per milliliter of capacity. The highest count was only 5,300 for the container, which is about 5.6 bacteria per milliliter of capacity.

The test for coliform organisms was applied to a large number of the containers at first but was discontinued because all containers examined gave negative results.

Wheaton, Lueck, and Tanner^{16*} examined 4,933 paraffined containers. About 80 percent yielded no colonies on agar plates, and of the remaining 20 percent over 90 percent gave fewer than 5 colonies per container.

Mudge and Foord,^{8*} using the needle-puncture method, reported that about 95 percent of the paraffined half-pint paper containers studied were sterile and a somewhat smaller percent of those of quart size were shown to be sterile.

More recently Rice^{9*} reported that: "from the broth sterility tests made on a total of 1,600 Pure-Pak containers selected uniformly from the several moisture-proofing treatments, it was found that approximately 80 percent of them were sterile."

It is well known that dairy equipment and utensils are not as a rule freed from all bacteria by the accepted methods of sterilization. For this reason various standards have been proposed for the maximum number of bacteria that can be present in utensils considered to be satisfactorily sterilized. In *Standard Methods for the Examination of Dairy Products*^{1*} it is stated that "quart bottles developing not more than 1000 colonies as determined by the method described above are generally considered satisfactory." According to this standard, about 99 percent of the 2,607 paper milk containers examined were satisfactorily sterilized. The results of the other investigators cited yielded similar results.

The bacterial condition of the paper milk containers examined might be compared with that of glass bottles as shown by the results of two surveys. Layson, Huffer, and Brannon^{7*} examined 454 glass bottles in twenty-five different milk plants. Two hundred ten, or 46.3 percent, failed to meet the standard of one bacterium per milliliter of capacity; 18 gave a bacterial count of over one million; and 63, or 14 percent, harbored coliform organisms.

The engineering section of the American Public Health Association made a survey of the bacteriological condition of sanitized milk bottles. The report of the survey states that only 56.9 percent of the glass bottles examined met a standard of one bacterium per milliliter of capacity.^{6*}

The paper milk containers examined in this study and those examined by the investigators cited were representative of the general

supply used in the milk industry. They were examined by the Standard Method and by even more exacting modifications of it. These examinations revealed that a large percentage of the containers were entirely free from bacteria and those not completely sterile yielded only a few bacteria. Several hundred containers were also tested for coliform organisms and not a single container was found to harbor these bacteria. All these results are a very strong proof that the general supply of paper containers sold to milk plants at the time of the study were in excellent sanitary condition. In order to keep these containers sanitary it was, of course, necessary to provide proper storage facilities for them at the milk plant.

Bacteriological Study of Pure-Pak Machine Operations

Adhesive as a source of bacteria. The adhesive used at the milk plant for sealing the sides and bottom of the Pure-Pak container was composed of tapioca starch, water, sodium nitrate (3 percent by weight), and Dovicide "A," a preservative. Seven lots of the adhesive were collected and five plates were made from each lot, using a dilution of 1 to 10. The number of colonies on the plates were as follows:

<i>Sample No.</i>	<i>Plate 1</i>	<i>Plate 2</i>	<i>Plate 3</i>	<i>Plate 4</i>	<i>Plate 5</i>
1.....	0	0	3	2	3
2.....	0	0	0	0	0
3.....	1	2	0	0	1
4.....	2	0	0	0	0
5.....	0	1	0	1	1
6.....	0	0	0	0	1
7.....	1	0	1	1	1

One pound of adhesive seals about 1,450 containers, so less than $\frac{1}{3}$ gram is used for each container. If the sample of adhesive giving the highest count, which was 16 bacteria per gram, was used, it would have added only about 5 bacteria to each container. When applied to the container, the adhesive is spread between the surfaces to be sealed, so that only a trace of it is exposed along the edge of the sealed seam. Moreover, when the container is paraffined, the exposed traces of the adhesive are covered. The possibility of bacterial contamination of milk by the adhesive is therefore very remote.

Hot paraffin as a source of bacteria. During the bottling operation a large number of Pure-Pak containers, which are not necessarily sterile, pass thru the paraffin bath. This affords an opportunity for bacteria to accumulate in the paraffin well. The Pure-Pak machine was used daily for about five months and during this period several samples of the hot paraffin were examined. The samples were invariably sterile.

Since the number of the containers paraffined at one time in the University plant was relatively small, the examination of the paraffin was also made in a commercial milk plant. In the first test at the com-

TABLE 5.—BACTERIAL CONDITION OF THE HOT PARAFFIN AT DIFFERENT TIMES DURING THE PARAFFINING PROCESS

Test 1	Colonies on plate	Colonies in agar tube
Time sample was taken		
11:50 a.m.—at start of paraffining.....	0	0
12:00 m.—after bottling whipping cream.....	0	0
12:05 p.m.—before bottling coffee cream.....	0	0
12:15 p.m.—after half pints of cream were bottled.....	0	0
12:30 p.m.—after all cream was bottled.....	0	0
12:50 p.m.—after adding 10 pounds of paraffin and before starting the bottling of milk.....	0	0
1:05 p.m.—after 15 minutes of bottling milk.....	0	0
1:25 p.m.—after adding 10 pounds of paraffin and 30 minutes of bottling milk.....	0	0
1:40 p.m.—after 45 minutes of bottling milk.....	0	0
1:55 p.m.—after adding 10 pounds of paraffin and 60 minutes of bottling milk.....	0	0
2:05 p.m.—bottling finished.....	0	0
2:05 p.m.—bottling finished (sample from drain).....	0	1

Test 2	Colonies on duplicate plates	Colonies in large tube	Growth in trip- licate tubes (+) or (-)
Time sample was taken			
11:20 a.m.—at start of paraffining.....	0-0	0	— — —
12:00 m.—after bottling whipping cream.....	0-3	0	— — —
12:25 p.m.—after bottling half-pints of coffee cream.....	0-1	0	— — —
12:45 p.m.—after bottling quarts of coffee cream.....	0-0	0	— — —
1:00 p.m.—after bottling milk.....	0-0	0	— — —
1:16 p.m.—after bottling milk.....	0-0	0	— — —
1:40 p.m.—after bottling milk.....	0-0	0	— — —
1:52 p.m.—after bottling milk.....	0-0	0	— — —
2:18 p.m.—bottling finished.....	0-0	0	— — —

mercial milk plant 10-ml. samples of the hot paraffin were withdrawn from the paraffin well every 15 minutes during the bottling of the milk. Five ml. were added to melted agar in petri dish and 5 ml. were added to a test tube containing nutrient agar.

In the second test at the commercial milk plant, which was performed several days later, the method of examination was modified. In making the agar plates, 10 ml. of the paraffin was spread over the bottom of each petri dish. Then agar was poured over the paraffin. Another sample of about 10 ml. of agar was introduced into large test tubes 7 inches long and an inch in diameter. The tubes were quickly rotated so the entire inner surface would be covered with the congealed paraffin, thus exposing a large surface of the paraffin to the broth which was then added. Also three regular-size test tubes containing nutrient broth were inoculated, each with 5 ml. of the paraffin at each sampling. All the cultures were incubated at 90° F. for two weeks.

In the first test all plates and all tubes except one remained free from any bacterial or mold growth (Table 5). The paraffin sample for the tube with the growth was taken from the drain of the paraffining unit.

In the second test one agar plate had 3 colonies and another had one

colony. None of the 36 test tubes with the nutrient broth developed bacterial, yeast, or mold growth.

The results of these tests of paraffin from the well of the Pure-Pak machine seem to indicate that during the paraffining process at 170° F. the paraffin remains practically sterile and therefore is not a source of contamination to the containers.

Cooling unit as a source of bacteria. After leaving the paraffin bath, the containers pass thru the cooling unit. Here the air is circulated by a fan in order to harden the paraffin quickly. To test the bacterial contamination of the air in the cooling unit, uncovered petri dishes containing nutrient agar were placed in the unit during the entire period of bottling. About 600 containers were filled and this required about one hour. After the bottling was finished, the petri dishes were covered and incubated. The numbers of colonies that developed on the exposed duplicate plates were as follows:

<i>Date of sample</i>	<i>Colonies on duplicate plates</i>	<i>Date of sample</i>	<i>Colonies on duplicate plates</i>
February 22.....	5- 2	March 5.....	13-11
February 23.....	5-13	March 6.....	3- 4
February 24.....	9- 5	March 9.....	1- 4
February 25.....	9- 6	March 10.....	7- 5
February 26.....	8- 6	March 11.....	4- 1
March 2.....	33- 7	March 12.....	6-11
March 3.....	14- 7	March 13.....	2- 6
March 4.....	4-28	April 17.....	2- 3

The average number of colonies per plate obtained during sixteen days of testing was 7.6. The opening of the Pure-Pak container has approximately the same area as that of the petri dish but the containers remain in the cooling unit only one minute. Calculations would therefore show that most of the containers were not contaminated by a single bacterium while passing thru the cooling unit.

Sealing knife as a source of bacteria. From the cooling unit the containers pass into the filling unit. After the containers are filled with milk, they are closed and sealed by an electrically heated knife. The hot knife is pressed against the edges of the container and the paraffin is melted. Then the edges are pressed together and the paraffin acts as an adhesive.

Tests were made to determine whether there was an accumulation of bacteria on the knife during the filling and sealing of 600 containers. The method consisted of wiping the knife with a sterile moist cotton swab both before and after the operation and then making counts of the bacteria removed each time.

The numbers of bacteria removed from the sealing knife by the sterile swab before and after the bottling operations on different days were as follows:

Date of sampling	Bacteria present		Date of sampling	Bacteria present	
	Before bottling	After bottling		Before bottling	After bottling
January 22.....	..	80	March 5.....	50	250
January 23.....	60	20	March 6.....	70	380
January 24.....	50	40	March 9.....	420	190
January 25.....	550	20	March 10.....	70	60
February 26.....	30	280	March 11.....	20	20
March 2.....	50	300	March 12.....	50	4 000
March 3.....	190	110	March 13.....	60	1 620
March 4.....	0	1 810			

The results of the tests indicate that the heat necessary to melt the paraffin was not sufficient to destroy all bacteria on the knife. In general, more bacteria were present after bottling than before, there being an average of 118 bacteria when the operation began and an average of 574 after sixteen days of bottling.

Even tho the number of bacteria on the knife increased during bottling operations, the contamination of milk would necessarily be small and would come mostly from the inside surface of the container along the sealed seam, which might be touched by the knife.

Bottling Machines as a Source of Contamination

The next step in this phase of the study was to determine the total bacterial contamination of milk in the three types of paper containers during the bottling operations.

Pure-Pak bottling process. In the case of the Pure-Pak machine, samples of milk were taken from: (1) the pasteurizing vat; (2) the machine reservoir; and (3) the filled and sealed container. The container samples were taken each day from the first three containers filled and then from every 25th container.

The results of six days of testing are given in Table 6. The average

TABLE 6.—EXTENT OF BACTERIAL CONTAMINATION OF REGULAR MILK AT DIFFERENT STAGES IN THE PURE-PAK BOTTLING PROCESS
(Average of agar-plate counts per milliliter of milk)

Sample	Average for 6 days	Sample	Average for 6 days
From pasteurizer		From filled bottles	
At start of bottling.....	216	3d.....	651
At end of bottling.....	146	25th.....	352
From machine reservoir		50th.....	274
At start of bottling.....	465	75th.....	238
In middle of bottling.....	181	100th.....	226
At end of bottling.....	205	125th.....	213
From filled bottles		150th.....	201
1st.....	785	175th.....	193
2d.....	742	200th.....	196
		225th.....	215
		250th.....	186

TABLE 7.—EXTENT OF BACTERIAL CONTAMINATION OF PRACTICALLY STERILE MILK AT DIFFERENT STAGES IN THE PURE-PAK BOTTLING PROCESS
(Agar-plate counts per milliliter of milk)

Sample	Test 1	Test 2	Sample	Test 1	Test 2	Sample	Test 1	Test 2
From can.....	4	5	From filled bottles			From filled bottles		
From reservoir...	7	5	6th.....	12	6	13th.....	..	11
From filled bottles			7th.....	12	11	14th.....	8	8
1st.....	62	52	8th.....	9	6	15th.....	9	4
2d.....	26	44	9th.....	..	6	16th.....	8	8
3d.....	17	7	10th.....	9	7	17th.....	11	15
4th.....	10	7	11th.....	12	5	18th.....	14	8
5th.....	16	8	12th.....	9	7	19th.....	16	8

bacterial count of the milk in the machine reservoir for six days was 465 per milliliter when it was first filled. The same milk back in the pasteurizing vat had given an average bacterial count of 249 per milliliter. In other words, the first milk took up an average of 249 bacteria per milliliter as it flowed from the pasteurizing vat, thru the pump, over an external tubular cooler, thru about 30 feet of sanitary pipe, and emptied into the reservoir. After about half the milk was bottled, the milk flowing thru the machine apparently took up no more bacteria from it, since the milk in the reservoir gave approximately the same bacterial count as it had done back in the pasteurizing vat.

The average bacterial count of milk in the first Pure-Pak container was 785 per milliliter, an average of 320 more bacteria per milliliter having been taken up by the milk since it flowed from the reservoir. The increase was undoubtedly due to the bacteria from the measuring cylinders. After about 100 containers were filled, the measuring cylinders did not appear to add any more bacteria to the milk, the bacterial counts of the milk in the filled container, in the reservoir, and in the pasteurizing vat being essentially the same.

An additional test was made to determine further the bacterial contamination of the milk in the Pure-Pak bottling process. In this test practically sterile milk was used and the milk did not pass thru any of the plant equipment but was poured directly from a milk can into the machine reservoir. The test was performed twice and each time a 5-gallon can of milk was bottled. Samples of the milk were taken from: (1) the milk can; (2) the reservoir; and (3) every container filled.

In both tests the bacterial counts in the first two Pure-Pak containers filled were higher than those in the milk from the reservoir (Table 7). The increase was small, however, and the counts in the bottles filled afterwards showed no appreciable increase.

Canco bottling process. To determine the extent to which the milk was contaminated by the machine filling the prefabricated Canco containers at the milk plant, samples were taken from: (1) pasteur-

TABLE 8.—EXTENT OF BACTERIAL CONTAMINATION OF MILK AT DIFFERENT STAGES IN THE CANCO BOTTLING PROCESS
(Average of agar-plate counts per milliliter of milk)

Sample	Average for 9 days	Sample	Average for 9 days
From pasteurizer.....	610	From last 15 bottles filled	
From cooler.....	985	586th.....	720
From machine bowl.....	1 255	587th.....	846
From first 15 bottles filled		588th.....	669
1st.....	1 481	589th.....	729
2d.....	1 450	590th.....	805
3d.....	1 340	591st.....	831
4th.....	1 483	592d.....	895
5th.....	1 256	593d.....	809
6th.....	1 676	594th.....	1 121
7th.....	1 220	595th.....	846
8th.....	1 246	596th.....	1 125
9th.....	1 251	597th.....	812
10th.....	1 421	598th.....	733
11th.....	1 331	599th.....	756
12th.....	1 244	600th.....	772
13th.....	1 365	Average.....	831
14th.....	1 356		
15th.....	1 470		
Average.....	1 386		

ized milk in the pasteurizing vat; (2) milk after it passed over the cooler; (3) milk in the machine reservoir; and (4) milk from the filled and sealed containers. Since the machine had fifteen filling valves, the first 15 and the last 15 of about 600 Canco containers filled each day were selected for the examination. Results of sampling on nine different days are given in Table 8.

The pasteurized milk on its journey from the pasteurizing vat to the filling machine passed thru a pump, over an external tubular cooler, thru about 60 feet of sanitary pipe in all, and finally was dropped into the machine reservoir. From here it was brought thru short pipes into the measuring cylinders and thence into the containers.

As the first pasteurized milk went from the pasteurizing vat thru a pump and about 20 feet of sanitary pipe and passed over the cooler, it picked up on the average 375 bacteria per milliliter. From the cooler the milk passed thru about 40 more feet of sanitary pipe into the bottling-machine reservoir. From this part of the equipment it picked up 270 bacteria per milliliter. The average bacterial count of the milk in the first 15 Canco containers filled was 1,386, an increase of 131 per milliliter over the count of the same milk in the reservoir. The average bacterial count of the milk in the last 15 Canco bottles filled was 831 per milliliter.

Sealright bottling process. To fill and cap the prefabricated Sealright containers, which are cone-shaped and have an opening similar to that of the glass bottle, the regular equipment for filling glass bottles was used with a special capping attachment.

In the Sealright bottling process the milk, after leaving the pasteur-

izing vat, passed thru a pump, over an external tubular cooler, thru about 25 feet of sanitary pipe, and into the filler bowl. Samples for bacteriological counts were taken from: (1) the pasteurizing vat after the milk was pasteurized; (2) the bottle-filler bowl; and (3) the filled

TABLE 9.—EXTENT OF BACTERIAL CONTAMINATION OF MILK AT DIFFERENT STAGES IN THE SEALRIGHT BOTTLING PROCESS
(Average of agar-plate counts per milliliter of milk)

Sample	Average for 6 days	Sample	Average for 6 days
From pasteurizing vat.....	657	From bottling bowl at end of bottling..	746
From bottling bowl at beginning of bottling.....	884	From last 6 bottles filled	
From first 6 bottles filled		501st.....	805
1st.....	899	502d.....	777
2d.....	939	503d.....	730
3d.....	893	504th.....	747
4th.....	916	505th.....	770
5th.....	837	506th.....	685
6th.....	881	Average.....	752
Average.....	894		
From bottling bowl halfway thru bottling	746		
From middle 6 bottles filled			
251st.....	803		
252d.....	761		
253d.....	838		
254th.....	844		
255th.....	765		
256th.....	701		
Average.....	785		

and sealed bottles. Approximately 600 quarts of milk were bottled each day. Since the filling machine had six valves, samples were taken daily from the first 6 bottles filled, 6 bottles filled when about half of the operation was completed (251st to 256th) and 6 bottles filled near the end (501st to 506th).

The average bacterial count of the milk for the six days was 657 in the pasteurizing vat, 894 in the first 6 Sealright containers filled, 785 in the 251st to 256th containers, and 752 in the last 6 containers (Table 9). The increase in the number of bacteria in the milk in the filled container over that in the pasteurizing vat was extremely small, showing that only a few bacteria were taken up by the milk from all the equipment, as well as from the Sealright container.

Results of tests with the three containers. The degree to which milk is contaminated by the equipment it contacts depends largely on the thoroughness with which the equipment is washed and sterilized. In this study the bottling machines and other equipment used in the milk plants were washed and disinfected in the regular way. The bactericidal treatment consisted of rinsing all parts of the bottling machine and other equipment with a sodium-hypochlorite solution containing about 100 ppm. of active chlorin. When any plant equipment is treated

in this way it is not as a rule completely sterile and may therefore add a few bacteria to the first flow of milk.

From the results of the tests made during the bottling process it can be concluded that the machines used in filling paper milk containers do not contaminate the milk to an extent that they present any special bacteriological problem.

Contamination of Milk by the Pouring Lip

The pouring lip of a container is the part with which the milk comes in contact when it is being poured from the container into another vessel. Naturally an unprotected pouring lip may become contaminated by harmful bacteria while the bottle is stored, transported, handled, and opened. Because of interest in the general bacteriological problem presented by the pouring lip of a milk container,^{3*} the glass milk bottle was included in this part of the study.

The procedure in making the tests was to contaminate the container on and around the lip and then to pour out some milk and examine it for the inoculating bacteria. Contamination was accomplished in two ways: (1) by spraying the bacterial suspension from an atomizer; and (2) by handling the containers and opening them with hands which had been dipped in the bacterial suspension. The bacterial suspension for all the tests was prepared from 48-hour agar slope culture of *Serratia marcescens*. The bacterial plate count of the suspension used was about 300 million per milliliter.

Pure-Pak containers. The Pure-Pak container used during the early period of this study was so designed that, in order to open it, it was necessary to loosen the wire staple and pull apart the seam which closed the top. To form the pouring lip, one side of the opening had to be bulged out. The easiest way to do this was to insert a finger and press outward. The milk was then poured, passing over the place where the finger had pressed. Since the opening was neither convenient nor sanitary, the container was later redesigned and improved, but the first test was made with the original container. Two other tests were made with the improved container, using quart, pint, and half-pint sizes.

Test 1 (original container).—In this test 3 Pure-Pak containers used as controls were opened with disinfected hands. The other Pure-Pak containers were not sprayed with the bacterial suspension but were handled and opened with contaminated hands. In opening 22 of the containers with contaminated hands, the finger was not pressed in to form the pouring lip but the containers were handled as follows: 6 were opened with special care not to touch the lip; 10 were picked up by the roof as the milkman picks them up; 6 were opened, partially emptied, closed, and stored at 40° F. for 24 hours, after which they

were opened with disinfected hands. Twelve containers were opened with contaminated hands, a finger being inserted to pull out the pouring lip. Of these 12 containers, 6 were simply opened and tested; 6 were opened, partially emptied, closed and stored at 40° F. for 24 hours, after which they were opened with disinfected hands.

Three samples of milk were taken from each bottle: (1) the first 100 ml. poured; (2) a 100-ml. sample after the container was half empty; (3) the last 100 ml. in the bottle. The samples were plated in

TABLE 10.—CONTAMINATION OF MILK BY THE INOCULATED POURING LIP OF PURE-PAK CONTAINERS

(A suspension of *Serratia marcescens* with a plate count of about 300 million bacteria per milliliter was used for inoculation)

Test 1 (original quart container handled with contaminated hands)	Positive and negative results of duplicate plates from—			
	First pouring	Second pouring	Third pouring	
Pouring lip not touched				
6 containers.....	- -	- -	- -	- -
Outside of pouring lip touched				
6 containers.....	- -	- -	- -	- -
1 container.....	+ +	- -	- -	- -
1 container.....	+ +	+ +	- -	- -
1 container.....	- -	- -	+ -	- -
1 container.....	+ +	+ -	+ -	- -
Inside of pouring lip touched				
6 containers.....	+ +	+ +	+ +	+ +
Outside of pouring lip touched and containers stored ^a				
6 containers.....	- -	- -	- -	- -
Inside of pouring lip touched and containers stored ^a				
3 containers.....	+ +	- -	- -	- -
1 container.....	+ +	- -	- -	- -
1 container.....	+ +	- -	- -	- -
1 container.....	+ -	- -	- -	- -
Opened with disinfected hands (controls)				
3 containers.....	- -	- -	- -	- -
<hr/>				
Test 2 (improved quart container)	Plate 1	Plate 2	Plate 3	Plate 4
Sprayed with bacterial suspension				
19 containers.....	-	-	-	-
1 container.....	-	+	-	-
Opened with contaminated hands				
2 containers.....	-	-	-	-
Opened with disinfected hands (controls)				
2 containers.....	-	-	-	-
<hr/>				
Test 3 (improved pint and half-pint container sprayed with bacterial suspension)	Plate 1	Plate 2	Plate 3	Plate 4
12 half-pint containers.....	-	-	-	-
6 pint containers.....	-	-	-	-

^aContainer was first partially emptied and then closed and stored at 40° F. for 24 hours before the milk sampled was poured over the lip.

duplicate, and after incubation the plates were examined for red colonies of *Serratia marcescens*.

Test 2 (improved container).—In the improved Pure-Pak container the opening is located on the slanting top, and the area around the opening is covered by a flap of paper fastened in place and extending about $\frac{1}{2}$ inch beyond the opening. Twenty-four quart-size improved containers were filled with milk. Twenty of them were sprayed around the closed opening with the bacterial suspension and then were opened with disinfected hands. Four containers were not sprayed, 2 of them being opened with disinfected hands and 2 with contaminated hands. Samples were taken from the first 100 ml. of milk poured from each container and four plates were prepared from each sample.

Test 3 (improved container).—Twelve half-pint and 6 pint-size improved Pure-Pak containers were filled with milk and then were sprayed with the bacterial suspension. They were opened with contaminated hands. Samples were taken from the first 100 ml. of milk poured from each container, and four plates were prepared from each sample.

Results of the three tests.—The results from Test 1 showed that the pouring lip of the original container was easily contaminated when opened with hands dipped in the bacterial suspension, and the milk in turn became contaminated when poured over the lip (Table 10). The first stream of milk that passed over the inoculated pouring lip did not remove all the organisms. The rest remained viable after the containers were kept 24 hours at 40° F. and some of them were picked up by the milk later poured over the lip.

In Test 2, which was made with the improved Pure-Pak container, only one red colony developed on one of the plates. The remaining 79 plates prepared from the 20 containers and 72 plates prepared from the 18 improved containers of Test 3 had no red colonies.

The results of the three tests indicate that the improved pouring lip of the Pure-Pak container is fully protected against any external contamination, including possible contamination from the hands when the container is opened.

Canco container. These containers also had two types of closures. The original cap overlapped the opening and extended to the edge of the container, covering the pouring lip on top but not protecting it against contamination from the side. The improved cap was similar to the original except that it extended farther and covered the side of the pouring lip as well as the top.

Test 1 (original container).—Twelve original Canco quart containers filled with milk were used as controls. They were not contaminated by spraying and were opened with disinfected hands. Thirty-six original Canco containers were opened and the milk was plated after

TABLE 11.—CONTAMINATION OF MILK BY THE INOCULATED POURING LIP OF CANCO CONTAINERS

(A suspension of *Serratia marcescens* with a plate count of about 300 million bacteria per milliliter was used for inoculation)

Method of handling containers	Positive and negative results when dilution on plate was—		
	1 ml.	.1 ml.	.01 ml.
Test 1 (original quart container)			
Handled by contaminated hands at filling machine			
12 containers.....	—	—	—
Sprayed with bacterial suspension just before entering filling machine			
12 containers.....	—	—	—
Filled with milk and then sprayed with bacterial suspension, first pouring			
6 containers.....	—	—	—
2 containers.....	+	+	+
2 containers.....	+	+	—
2 containers.....	+	—	—
Filled with milk and then sprayed with bacterial suspension, second pouring			
5 containers.....	+	—	—
5 containers.....	+	+	—
1 container.....	+	+	+
1 container.....	+	+	—
Not contaminated and opened with disinfected hands (controls)			
12 containers.....	—	—	—
Test 2 (improved quart container contaminated by spraying)			
First pouring			
15 containers.....	—	—	..
5 containers.....	+	+	..
4 containers.....	+	—	..
Second pouring			
19 containers.....	—	—	..
3 containers.....	+	+	..
2 containers.....	—	+	..

the containers had been contaminated as follows: 12 were handled in the usual way but by contaminated hands as they came from the filling machine; 12 closed containers were sprayed before filling and then the containers were passed thru the filling machine; 12 containers were filled and then sprayed on top and around the pouring lip.

Test 2 (improved container).—In this test 24 quart-size Canco containers with the improved pouring lip were filled with milk and then were contaminated by spraying. The containers were opened, about a half pint of milk was poured out and plates were prepared from it.

Results of the two tests.—In the first test no red colonies developed on plates from the containers which were handled with contaminated hands at the filling machine or from the containers which were sprayed before they were filled (Table 11). Of the 12 containers sprayed on and around the pouring lip before being filled, 7 yielded red colonies.

In Test 2, 9 containers out of 24 yielded red colonies.

The results from the two tests show that when the pouring lip of a Canco container is heavily inoculated, the milk becomes contaminated with some of the inoculating organisms as it is poured from the container. This is true of both the original and the improved pouring lip.

Sealright container. In this test 12 quart-size Sealright containers were filled with milk and then closed with a paper cap which covered the outside rim of the opening to a depth of about $\frac{1}{4}$ inch.

TABLE 12.—CONTAMINATION OF MILK BY THE INOCULATED POURING LIP OF QUART SEALRIGHT CONTAINERS

(A suspension of *Serratia marcescens* with a plate count of about 300 million bacteria per milliliter was used for inoculation)

Method of handling containers	Positive and negative results of triplicate plates from—					
	First pouring			Second pouring		
Sprayed with bacterial suspension						
2 containers.....	+	+	+	-	-	-
1 container.....	+	+	+	-	-	-
1 container.....	+	+	+	+	-	-
1 container.....	+	+	-	-	+	-
1 container.....	+	+	-	+	+	-
Opened with contaminated hands						
2 containers.....	+	-	-	-	-	-
2 containers.....	-	-	-	-	-	-
Not sprayed, and opened with disinfected hands (controls)						
2 containers.....	-	-	-	-	-	-

Two of these containers used as controls were not sprayed and were opened with disinfected hands. Ten containers were contaminated, 6 being sprayed with the bacterial suspension and 4 being opened with contaminated hands. The caps were removed from the containers and samples of milk taken from the first stream of milk poured and again from the last stream poured. Agar plates were prepared from the samples, and after incubation they were examined for red colonies.

The results (Table 12) indicate that when the Sealright containers were closed with the cap described and were contaminated around the pouring lip or when the cap was removed with contaminated hands, the milk picked up some of the contaminating organisms as it was poured over the lip.

Glass milk bottles. Forty quart glass bottles were filled with milk and closed with the regular paper cap or disk. Three of these bottles used as controls were not contaminated with the bacterial suspension and were opened with disinfected hands. Thirty-four bottles were contaminated with hands dipped in the bacterial suspension and were handled as follows: 6 were opened carefully so that the pouring lip was not touched; 10 were picked up by the top, as is usually done by the delivery man; 6 were opened in the usual way, with the fingers

TABLE 13.—CONTAMINATION OF MILK BY THE INOCULATED POURING LIP OF QUART GLASS BOTTLES

(A suspension of *Serratia marcescens* with a plate count of about 300 million bacteria per milliliter was used for inoculation)

Method of handling containers	Positive and negative results of duplicate plates from—					
	First pouring		Second pouring		Third pouring	
Opened with contaminated hands but pouring lip not touched						
3 containers.....	-	-	-	-	-	-
1 container.....	-	-	+	-	-	-
1 container.....	+	+	-	-	-	-
1 container.....	+	+	+	+	+	+
Opened with contaminated hands and outside of pouring lip touched						
9 containers.....	+	+	+	+	+	+
1 container.....	-	-	-	-	-	-
Opened with contaminated hands and inside of pouring lip touched						
6 containers.....	+	+	+	+	+	+
Opened with contaminated hands touching outside of pouring lip and container stored*						
4 containers.....	+	+	+	+	+	+
2 containers.....	+	-	-	-	-	-
Opened with contaminated hands touching inside of pouring lip and container stored*						
6 containers.....	+	+	+	+	+	+
Pouring lip sprayed with bacterial suspension, then wiped with sterile towel						
4 containers.....	+	+	+	+	+	+
Opened with disinfected hands (controls)						
3 containers.....	-	-	-	-	-	-

*Container was first partially emptied and then closed and stored at 40° F. for 24 hours before the milk sampled was poured over the lip.

resting on the pouring lip; 6 were picked up by the top, held a few seconds, opened and partially emptied, then closed and stored at 40° F. for 24 hours, after which they were opened with disinfected hands and tested; 6 were opened in the usual way, with the fingers resting on the pouring lip, then they were partially emptied, closed, and stored away at 40° F. for 24 hours, after which they were opened with disinfected hands and tested. Three bottles not handled with contaminated hands were sprayed with the bacterial suspension, the pouring lip was wiped with a sterile towel, and the bottles were opened with disinfected hands.

When each bottle was opened, three samples of milk were taken: (1) the first 100 ml. poured; (2) a 100-ml. sample after the container was half empty; (3) the last 100 ml. in the bottle. Plates were made from each sample, and after incubation the plates were examined for red colonies. The results are given in Table 13.

The results of these tests show that the unprotected pouring lip of a glass bottle is likely to be contaminated when the bottle is picked

up and held by the top. It can also be contaminated when the regular paper cap is being removed. Simply wiping the pouring lip does not remove all bacteria. The inoculating organisms survived after the bottles were stored for 24 hours at 40° F. and they continued to contaminate the milk that was poured over the lip.

Penetration of Paraffined Walls by Bacteria

Since paper containers may be exposed to external contamination after they are filled with milk, the question has been raised whether bacteria can pass thru the paraffined walls into the milk. The following experiment was designed to throw light on this question.

Paraffined paper containers were filled with milk and then submerged in a suspension of *Serratia marcescens* in the following way: 8 were submerged up to the pouring lip and held there for 24 hours, 4 of these at 40° F. and the other 4 at 70° F.; 4 were submerged up to the pouring lip for 48 hours at 40° F.; 6 were completely submerged at 40° F., 4 of these for 24 hours and 2 for 48 hours.

After each container was taken from the suspension it was sterilized by being submerged in a strong chlorin disinfecting solution. It was then opened, a sample of milk was taken, and four agar plates were poured from it. These were examined for red colonies after incubation.

None of the plates showed the *Serratia marcescens* colonies of the bacterial suspension. According to these results, bacteria do not pass thru the paraffined walls of paper containers from a surrounding contaminated medium into the milk.

Bactericidal Action of Paraffining

Paper containers are paraffined primarily for the purpose of making them impervious to water. Since paraffining must be done at relatively high temperatures, the process also involves bactericidal action, the nature of which was considered in this phase of the study. Tests were conducted with small plaques of paper in the laboratory and with regular-size containers in the milk plant.

Laboratory study. The first set of tests made in the laboratory were carried out in the following manner. Plaques measuring $\frac{1}{2}$ by $2\frac{1}{2}$ inches were cut from the paperboard used for making the containers and were impregnated with bacteria by being dipped in a bacterial suspension. The suspension was prepared by putting a 24-hour agar slope growth of the organism into sterile water to which had been added one percent of sterile skim milk. After the plaques were dry, which required about fifteen minutes, they were submerged in the melted paraffin. The temperature of the paraffin and the length of time the plaques were held in it were varied. Three different organisms

were used in the study: *Serratia marcescens*, *Escherichia coli*, and *Staphylococcus aureus*.

The method used for determining whether viable organisms survived consisted of dropping the paraffined plaques of paperboard into test tubes containing nutrient liquid media and then incubating the test tubes.

The plaques inoculated with *Serratia marcescens* were put in test tubes containing a mixture of 85 percent water and 15 percent skim-milk, and were incubated at 70° F. The organism grew readily in this mixture and produced a deep red color. The development of red color on the plaques or in the mixture was considered evidence that *Serratia marcescens* organisms survived the paraffining.

The plaques inoculated with *Escherichia coli* were put in fermentation tubes containing brilliant green bile broth and were incubated at 100° F. The development of gas was considered evidence that *Escherichia coli* organisms survived the paraffining.

The plaques inoculated with *Staphylococcus aureus* were put in test tubes containing regular nutrient broth and were incubated at 100° F. When any growth occurred, the broth was examined with a microscope and subcultures were made to ascertain whether the growth was that of the inoculating organism.

In the test with *Serratia marcescens* each plaque was inoculated with a million organisms. The plaques that were paraffined at 170° F. for 45 seconds and at 180°, 190°, and 200° F. for 30 seconds, and those paraffined at 210° F. for 20 seconds did not yield any growth in the nutrient liquid (Table 14). When the plaques were exposed for a shorter time to the same temperatures, viable *Serratia marcescens* organisms were occasionally found on some plaques.

Each plaque of *Escherichia coli* in this test was inoculated with 8 million organisms. The plaque paraffined at 190° F. for 45 seconds, and those paraffined at 200° F. for 20 seconds developed no *Escherichia coli* growth in the fermentation tubes (Table 14). Some plaques paraffined at these temperatures for a shorter time caused gas to form in the fermentation tubes. Of the 6 plaques paraffined at 180° F. for 120 seconds and the 6 paraffined at 185° F. for 60 seconds, 1 from each group yielded viable *Escherichia coli* organisms and the remaining 5 yielded no growth.

Staphylococcus aureus is more resistant to heat than the other two organisms used. In order to kill all the staphylococcus organisms by paraffining, a temperature of 200° F. for not less than 2 minutes was necessary (Table 14).

The tests made up to this point for bacteria surviving the paraffining process yielded only positive or negative results. In succeeding tests the method was modified so that the percentage reduction of bacteria brought about by the paraffining process could be computed.

TABLE 14.—DESTRUCTION OF BACTERIA BY PARAFFINING PAPER PLAQUES INOCULATED WITH SUSPENSIONS INDICATED

Temperature of paraffin and number of plaques	Positive and negative results when plaques were exposed to paraffin—					
	10 seconds	20 seconds	30 seconds	45 seconds	60 seconds	120 seconds
Test with <i>Serratia marcescens</i> (1 million per plaque)						
160° F.						
2 plaques	+	+	+	+	+
2 plaques	+	+	+	+	+
1 plaque	+	+	+	—	—
1 plaque	+	+	—	—	—
170° F.						
4 plaques	+	+	+	—	—
1 plaque	+	+	—	—	—
1 plaque	+	—	—	—	—
180° F.						
3 plaques	+	+	—	—	—
1 plaque	—	+	—	—	—
2 plaques	—	—	—	—	—
190° F.						
1 plaque	+	+	—	—	—
1 plaque	+	—	—	—	—
4 plaques	—	—	—	—	—
200° F.						
1 plaque	+	+	—	—	—
1 plaque	—	+	—	—	—
4 plaques	—	—	—	—	—
210° F.						
2 plaques	+	—	—	—	—
4 plaques	—	—	—	—	—
Test with <i>E. coli</i> (8 million per plaque)						
180° F.						
1 plaque	+	+	+	+	+	+
2 plaques	+	+	+	+	+	—
1 plaque	+	+	+	+	+	—
2 plaques	+	+	+	—	—	—
185° F.						
1 plaque	+	+	+	+	+	—
2 plaques	+	+	+	+	—	—
2 plaques	+	+	+	—	—	—
1 plaque	+	—	—	—	—	—
190° F.						
3 plaques	+	+	+	—	—	—
1 plaque	+	—	—	—	—	—
2 plaques	—	—	—	—	—	—
200° F.						
2 plaques	+	—	—	—	—	—
4 plaques	—	—	—	—	—	—
Test with <i>Staphylococcus aureus</i> (10 million per plaque)						
160° F.						
6 plaques	+	+	+	+	+	+
170° F.						
6 plaques	+	+	+	+	+	+
180° F.						
6 plaques	+	+	+	+	+	+
190° F.						
6 plaques	+	+	+	+	+	+
200° F.						
1 plaque	+	+	+	+	+	+
2 plaques	+	+	+	+	+	—
3 plaques	+	+	+	+	—	—

Plaques weighing 2 grams and measuring about 2 by 4½ inches were inoculated by being dipped in a bacterial suspension of *Serratia marcescens*. Part of the inoculated plaques were paraffined and part were left unparaffined and the results were compared.

The plaques that were paraffined were submerged at 170° F. for 20 seconds. This combination of time and temperature was considered comparable to that used in the paraffining unit of the Pure-Pak machine (page 416). Some of these plaques were paraffined immediately after they were inoculated and while still moist and others were allowed to dry for 30 minutes at 75° F. before paraffining. As soon as the coating hardened, the paraffined plaques were reduced to pulp in 200 ml. of water. Ten agar plates, each receiving 2 ml., were poured from the pulp mixture. The plaques that were not paraffined were reduced to a pulp and plated in the same manner. All plates were incubated for 3 days at 75° F. and the *Serratia marcescens* colonies were then counted.

In Test 1, 6 plaques were inoculated by being dipped in the bacterial suspension. One of these plaques was reduced to pulp as soon as inoculated, and it was not paraffined. The plate counts from the pulp-and-water mixture indicated that each of the plaques had been inoculated with about 72,570 organisms. Two of the 6 plaques were paraffined immediately after they were dipped in the suspension and while they were still wet. One of these wet plaques yielded a total count of 10 bacteria and the other 20 bacteria. Thus the reduction in bacteria on the plaques caused by paraffining was more than 99.97 percent. Three of the 6 plaques were allowed to dry at room temperature for 30 minutes. One plaque that was not paraffined yielded a plate count of 2,520 bacteria, drying causing a reduction of about 96.5 percent. The 2 plaques that were allowed to dry and then were paraffined yielded no colonies. Apparently all the inoculating organisms were destroyed by the paraffining process.

In Test 2 each of 6 plaques was inoculated with 57,850 organisms. The plaque that was dried but not paraffined yielded a plate count of 3,620 bacteria, drying bringing about a reduction of 93.8 percent. The 2 plaques paraffined immediately after inoculation yielded no colonies.

In Test 3 each of 6 plaques was inoculated with 82,110 organisms. The one dried but not paraffined gave a count of 1,280, a 98.5-percent reduction in bacteria being caused by drying. In 4 plaques there was a 100-percent reduction brought about by paraffining, none of these paraffined plaques yielding colonies.

In Test 4 each of 6 plaques was inoculated with 23,010 organisms. The plaque that was dried but not paraffined gave a plate count of 1,260, a reduction of 94 percent caused by drying. None of the paraffined plaques yielded colonies.

The results from the four tests just described show that when

the inoculated plaques were allowed to dry for 30 minutes at room temperature, over 90 percent of the inoculating organisms disappeared. When the plaques were heavily inoculated and were paraffined at 170° F. for 20 seconds, all inoculating organisms were destroyed on 13 plaques and 99.97 percent on the remaining 3 plaques.

Milk-plant study. Since the Canco and Sealright containers were paraffined at the carton factories, the study of paraffining at the University milk plant was confined to the Pure-Pak container.

The machine that paraffined the Pure-Pak containers was so constructed that its speed could not be easily changed, but the temperature of the paraffin could be varied as desired. All the containers in the following experiment were therefore paraffined for the same length of time but the temperature was varied. As the containers were passed thru the paraffining unit, they were exposed to the hot air above the paraffin for 3.75 seconds, then they were submerged in the hot paraffin for 11.25 seconds, and then left in the hot air above the paraffin for 13.12 seconds while the excess paraffin was drained.

The procedure in these experiments was as follows: The partially fabricated containers were inoculated and then passed thru the machine. In the first unit the containers were formed and their bottoms sealed with an adhesive. In the second unit they were paraffined. From here they passed into the refrigerating unit, where the paraffin was allowed to harden. From the refrigerating unit they passed thru the filling unit and finally thru closing and sealing units. No milk was put in the containers. The sealed containers were taken to the laboratory, where they were examined for viable inoculating organisms.

For inoculating purposes a strain of highly colored *Serratia marcescens* was used. The organism grew readily on the standard nutrient agar and produced deep red colonies which were easily recognized.

To determine whether any of the inoculating organisms survived the paraffining, 25 ml. of nutrient agar were poured into each container. After gently agitating the container to bring the entire inside surface in contact with the agar, the containers were placed in the incubator. After incubation, the agar was examined for the characteristic red colonies of *Serratia marcescens*.

Three experiments were conducted with quart-size Pure-Pak containers, using heavy, moderate, and light contamination.

Heavy contamination.—The first experiment consisted of impregnating the containers heavily with bacteria, both inside and outside, by completely submerging them in the bacterial suspension for about 5 seconds. Each container absorbed about 5 ml. of the suspension.

Of the 121 containers paraffined at 170° F., 24 were positive, that is, they yielded one or more colonies of the inoculating bacteria, and 97 were negative (Table 15). Of the 62 containers paraffined at 175° F., 2 were positive. Of the 96 containers paraffined at 180° F.,

TABLE 15.—DESTRUCTION OF BACTERIA BY PARAFFINING PAPER MILK CONTAINERS THAT HAD RECEIVED A HEAVY CONTAMINATION OF *Serratia marcescens*

Plate count of suspension used for inoculation	Number of containers	Number of negative and positive containers when paraffining temperature was—									
		170° F.		175° F.		180° F.		185° F.		190° F.	
		-	+	-	+	-	+	-	+	-	+
<i>millions</i>											
90.0.....	20	8	2	7	3
22.0.....	60	12	0	12	0	12	0	12	0	12	0
18.0.....	48	23	1	24	0
13.0.....	39	18	21
2.0.....	75	24	1	24	1	25	0
1.8.....	36	36	0
.1.....	75	24	1	25	0	25	0
Total.....	353	97	24	60	2	92	4	62	0	12	0

4 were positive. All the containers paraffined at 185° and 190° F. were negative.

Moderate contamination.—In the above test, submerging the containers in the bacterial suspension caused them to warp to such an extent that the machine became jammed. Furthermore such extremely heavy contamination would never occur in regular milk plant operations and hence it was not a fair test. In the second experiment the containers were therefore given a moderate contamination. The operator dipped his hands in the bacterial suspension and without drying them held the container with one hand and pushed the other hand in and out, thus contaminating the inner surface.

Eighteen of the 300 containers paraffined at 160° F. were positive, that is, one or more colonies of the inoculating bacteria developed on the agar; the rest were negative (Table 16). Five of the 400 containers paraffined at 170° F. were positive, as were 6 of the 600 paraffined at

TABLE 16.—DESTRUCTION OF BACTERIA BY PARAFFINING PAPER MILK CONTAINERS THAT HAD RECEIVED A MODERATE CONTAMINATION OF *Serratia marcescens*

Plate count of suspension used for inoculation	Number of containers	Number of negative and positive containers when paraffining temperature was—											
		160° F.		170° F.		180° F.		185° F.		190° F.		200° F.	
		-	+	-	+	-	+	-	+	-	+	-	+
<i>millions</i>													
5,000.0.....	300	91	9	99	1	100	0
250.0.....	300	91	9	99	1	98	2
120.0.....	400	97	3	96	4	91	9	98	2
10.0.....	600	200	0	200	0	200	0
2.5.....	300	100	0	100	0	100	0
Total.....	1 900	282	18	395	5	594	6	200	0	291	9	98	2

TABLE 17.—DESTRUCTION OF BACTERIA BY PARAFFINING PAPER MILK CONTAINERS THAT HAD RECEIVED A LIGHT CONTAMINATION OF *Serratia marcescens*

Plate count of suspension used for inoculation	Number of containers	Number of negative and positive containers when paraffining temperature was—					
		170° F.		180° F.		190° F.	
		—	+	—	+	—	+
<i>millions</i>							
200.....	600	200	0	200	0	199	1
100.....	300	100	0	100	0	100	0
50.....	300	100	0	100	0	100	0
Total.....	1 200	400	0	400	0	399	1

180° F., 9 of 300 paraffined at 190° F., and 2 of the 100 paraffined at 200° F. The 200 containers paraffined at 185° F. were all negative.

Light contamination.—In the third experiment the operator, after dipping his hands in the bacterial suspension, handled the containers just as in regular operations in the plant. He picked up about 15 at a time from the shipping carton and placed them in the rack on the machine. From here they went automatically thru the machine.

Under this light contamination, the 400 containers paraffined at 170° F. were all negative, as were also the 400 paraffined at 180° F. (Table 17). Of the 400 paraffined at 190° F. all but one were negative.

Summary of experiments at the milk plant.—From the results of the foregoing experiments it appears that only a few organisms survive the paraffining process even after heavy contamination. In the heaviest contamination about 110 million organisms were deposited per square inch of surface. When the contamination was light, or such as might occur in the regular handling of containers just before paraffining, the containers were free from the inoculating organisms after paraffining. In the last experiment all 1,200 containers that had been contaminated were free from the inoculating organism after they were paraffined with the exception of one container. The one colony present in this container paraffined at 190° F. was undoubtedly due to contamination subsequent to paraffining.

Standards for paraffining. Paper milk containers are paraffined to make them impervious to water and rigid. At what temperature the paraffin should be applied and how long the containers should be exposed to it to make them sanitary have been debatable questions.

There appear to be two phases to the bactericidal action of paraffining. In the first place, the heat in the paraffin destroys bacteria, the percentage of bacteria killed depending upon the temperature of the paraffin and the length of time the containers are exposed to it. In the second place, the hot paraffin appears to cover or imprison bacteria

as it penetrates the body of the paper and forms a coating on the surface. The paper is not sterile and most of the bacteria found in it are in the spore stage, in which they cannot be destroyed by the heat of the paraffin. Yet a large percentage of the paraffined containers have been shown to be entirely free from bacteria, as determined by the Standard Method used for that purpose. Thus the combined action of the paraffin, the destruction of most of the bacteria by heat and the imprisonment of those that survive the heat, yields containers which are sterile in most cases, and the occasional container that is not entirely sterile has only one or two bacteria in it.

Experiments with paraffining paper milk containers have shown that temperatures much higher than about 170° F. are not practical because they do not permit the containers to retain enough paraffin to make them rigid. If the temperature of the paraffin is too low, too much paraffin will adhere to the paper. Obviously the time and temperature of the paraffining process must be such as to produce a satisfactory container from a physical standpoint.

PART II

CERTAIN PHYSICAL ASPECTS OF PAPER MILK CONTAINERS

When paper containers were first introduced, there were some questions as to their practicability for commercial operations. Tests were therefore made of certain of their physical characteristics thought to have a bearing on their general usefulness.

Since the Pure-Pak container is partially fabricated at the milk plant, it was possible to study it in more detail than either the Canco or the Sealright bottle. For this reason many of the tests were made only on the Pure-Pak container. Also, whenever it was apparent that the results of tests on one bottle would apply to all three, the tests were not repeated. Quart containers were used in all tests.

Amount of Paraffin Used in Waterproofing Container

In ascertaining the amount of paraffin that adheres to quart paper containers, 25 Pure-Pak bottles of both the heavy- and light-weight paper were used and were covered with paraffin of both a high and a low melting point. The paraffin was applied at five different temperatures: 160°, 170°, 180°, 190°, and 200° F. The bottles were weighed before and after passing thru the machine unfilled and an allowance of .08 gram was made for the weight of the wire staple.

The amount of paraffin adhering to the paper was found to be directly related to the temperature of application, more paraffin being held by the paper at the lower temperature (Table 18). The thickness

TABLE 18.—AMOUNT OF PARAFFIN NEEDED TO COAT LIGHT AND HEAVY PAPER CONTAINERS WHEN APPLIED AT DIFFERENT TEMPERATURES
(Averages of 25 containers)

Thickness of paper <i>inches</i>	160° F.		170° F.		180° F.		190° F.		200° F.		
	Melting point of paraffin ° F.	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	Weight Percent*	
.016.....	125-127	14.72	30.08	14.32	29.40	14.18	29.70	13.52	28.20	13.48	28.70
.016.....	135-137	14.50	30.10	14.70	30.30	14.00	29.10	13.82	29.00	13.50	28.70
.019.....	125-127	14.80	26.80	14.35	26.10	14.12	25.70	13.33	24.90	12.52	23.60
.019.....	135-137	15.62	28.00	14.80	26.82	14.44	26.20	13.40	24.80	12.30	23.00

*Percent paraffin was of total weight of container.

TABLE 19.—TEMPERATURE AT WHICH PARAFFIN COATING ON HEAVY AND LIGHT PAPER CONTAINERS BEGAN TO MELT
(Averages of 4 containers)

Thickness of paper <i>inches</i>	Temperature at which coating began to melt	
	Melting point of paraffin ° F.	Trial 1 Trial 2 ° F.
.016.....	125-127	120.2 121.1
.016.....	135-137	131.0 131.0
.019.....	125-127	120.2 121.1
.019.....	135-137	131.0 131.0

of the paper and the melting point of the paraffin had no significant effect on the amount of paraffin needed to coat the container.

Temperature at Which Paraffin Melts From Container

To determine at what temperature paraffin is likely to melt and run into the milk in the container, two sets of tests were made. In these tests light (.016-inch paper) and heavy (.019-inch paper) Pure-Pak containers were treated with paraffin of both a high (135° to 137° F.) and a low 125° to 127° F.) melting point. The paraffined bottles left unfilled were placed in an electric hot-air incubator at 75° F. The temperature was raised at the rate of 2 degrees a minute until the melted paraffin could be detected on the plain white paper on which the bottles were standing. Averages of samples from four bottles were taken.

Results with the Pure-Pak container showed that when the container was covered with paraffin of a low melting point, the paraffin would not begin to melt and get into the milk until the surrounding air reached a temperature of a little over 120° F.; when covered with paraffin of a high melting point a temperature of 131° F. was necessary before the paraffin started to melt into the milk (Table 19, page 453). The weight of the paper did not affect the temperature at which the paraffin melted from the bottle.

Factors Affecting Rigidity of Filled Container

A certain degree of rigidity in the walls of paper milk containers is necessary to prevent excessive bulging and possible splitting of the glued seams. Because it was thought that moisture absorption might be related to bulging, a test was made to determine whether the walls of the paper container take up moisture from the contents during storage. Other tests were made to determine the relation of bulging to such factors as the weight of the paper from which the container was made and the melting point of the paraffin applied, time and temperature of storage, and amount of lactic acid in the product put in the bottle.

Moisture absorption. To determine whether moisture is absorbed by the walls of a filled container during storage, the following experiment was performed.

Empty Pure-Pak containers were weighed after the application of paraffin. They were then filled with skim milk and one lot stored at 40° F. and the other lot at 72° F. Duplicate containers of both lots were taken out of storage after 8, 24, 54, and 72 hours. After being stored the designated time, each container was emptied, washed with distilled water, air-dried, and weighed. The gain in the weight of the container during storage was taken as the measure of the amount of moisture absorbed. Results are given in Table 20.

TABLE 20.—MOISTURE ABSORBED BY PAPER CONTAINERS WHEN FILLED WITH SKIMMILK (Averages of 2 containers)

Time stored	Containers with paraffin melting point of 125°-127° F. stored at—		Containers with paraffin melting point of 135°-137° F. stored at—	
	40° F.	72° F.	40° F.	72° F.
Moisture absorbed by light-weight containers (.016 inch)				
hours	gm.	gm.	gm.	gm.
8.....	.510	.580	.525	.570
24.....	1.045	1.055	.870	.010
54.....	1.535	1.715	1.535	1.655
72.....	1.830	1.960	1.955	2.070
Moisture absorbed by heavy containers (.019 inch)				
hours	gm.	gm.	gm.	gm.
8.....	.550	.835	.610	.925
24.....	1.135	1.470	1.525	1.510
54.....	2.180	2.455	1.900	2.355
72.....	2.285	2.510	2.435	2.650

Apparently less moisture is absorbed by light-weight paper containers than by containers made from the heavier paper, but there is little difference that can be traced to the melting point of the paraffin with which containers are covered. The amount of moisture absorbed by any one container is dependent upon the time and temperature of storage.

To determine at what point the moisture penetrates the paraffin, several Pure-Pak containers were completely filled with a solution of 1-percent methylene blue in water. After standing 30 minutes, the dye was poured out and the containers were cut open and examined. Usually there was a slight degree of penetration along the scored corners and seams, as indicated by discolored spots. The amount of discoloration varied with individual bottles, the unscored ones showing the least penetration. Studies were made in which potassium iodide and Jensen violet were each substituted for the methylene-blue solution, and similar results were obtained.

Time and temperature of storage. In the experiment designed to test the effect of temperature of storage on bulging, three lots of 13 containers each were stored under different temperature conditions. Eleven of the containers from each lot were from shipments prepared for commercial dairies and were all of the light-weight stock. The other two containers from each lot were unprinted, one from light-weight stock and the other from the heavier stock. All containers were treated with paraffin having a melting point of 125° to 127° F. They were all filled with sweet whole milk at 42° F. and then placed in cases and handled as follows:

The first lot, which was measured daily, was stored at 40° F. for 5 days. After the bottles had been in the fiber cases for 18 hours they were taken out and put on the shelf.

The second lot was kept in the cases and stored for 96 hours at 40° F. The bottles were then unpacked and placed on a table at a temperature of 72° F.

The third lot, after being stored in the case at 40° F. for 96 hours was removed and put on a table at 102° F.

The diameters of the sides of the container were measured with a caliper at the points where the bulging was greatest. Measurements were taken before and after storage, any increase in diameter being considered the measure of the bulging. Diameter 1 was taken from the two sides which provide the tucked-in top and Diameter 2 from the sides that extend to the stapled top.

TABLE 21.—BULGING OF PAPER CONTAINERS FILLED WITH SWEET WHOLE MILK AND STORED AT DIFFERENT TEMPERATURES FOR VARYING PERIODS (Expressed in 32d's of an inch)

Container No.	Expansion when stored at 40° F. for—							
	24 hours		48 hours		96 hours		120 hours	
	Diam. 1	Diam. 2	Diam. 1	Diam. 2	Diam. 1	Diam. 2	Diam. 1	Diam. 2
1.....	2	6	2	6	2	6	2	10
2.....	2	6	2	8	2	8	2	10
3.....	2	4	2	4	2	6	2	6
4.....	2	4	2	4	2	4	2	6
5.....	2	6	4	8	2	8	2	10
6.....	2	4	4	6	2	6	2	8
7.....	2	6	4	6	4	6	4	8
8.....	2	4	2	4	2	4	2	6
9.....	2	2	2	2	2	4	2	6
10.....	2	4	2	4	2	4	4	6
11.....	2	4	2	6	2	6	4	8
12.....	2	6	2	10	2	10	2	16
13.....	2	2	2	2	4	4	4	6

Container No.	Expansion when stored at 40° F. for 96 hours, then at 72° F. for 24 hours		Container No.	Expansion when stored at 40° F. for 96 hours, then at 102° F. for 24 hours	
	Diam. 1	Diam. 2		Diam. 1	Diam. 2
1A.....	2	2	1B.....	6	10
2A.....	0	6	2B.....	4	8
3A.....	2	6	3B.....	6	8
4A.....	2	6	4B.....	10	8
5A.....	0	6	5B.....	2	10
6A.....	2	6	6B.....	10	12
7A.....	2	4	7B.....	10	10
8A.....	2	2	8B.....	6	8
9A.....	0	2	9B.....	10	12
10A.....	2	2	10B.....	4	8
11A.....	0	4	11B.....	4	6
12A.....	0	8	12B.....	4	16
13A.....	6	4	13B.....	4	8

All the containers of the first lot had expanded $\frac{1}{16}$ inch while they were in the sealed cases for 18 hours. The containers of the third lot, which were first stored at 40° and then at 102° F., showed the most bulging (Table 21). They took on an oily appearance on the outside wall above the line of the milk. There was very little difference in the bulging of the containers made from light- and heavy-weight stock, and the 11 commercial containers tested were fairly uniform in their standing-up qualities. In all bottles the greater amount of expansion usually took place on the sides that extend to the stapled top (at Diameter 2), these sides apparently receiving less support than the sides extending to the tucked-in top.

Similar results were obtained when all the containers were coated with paraffin having a high melting point.

Lactic acid in contents. In commercial use at the University creamery the paper containers filled with cultured milk did not stand up so well as those filled with sweet milk or cream. Cultured milk caused the sides to shrivel in some styles of container and to bulge in others.

TABLE 22.—LEAKAGE IN PAPER CONTAINERS AS AFFECTED BY WATER SOLUTION OF LACTIC ACID
(Containers stored at 80° F. for six days)

Kind of container and percent of lactic acid	Days elapsing before leakage occurred	Place where leakage occurred
Pure-Pak		
.17 percent.....	No leakage ^a
.475 percent.....	4	At bottom along glued flap
.85 percent.....	2	At bottom along glued flap
Canco		
.17 percent.....	No leakage ^a
.475 percent.....	3	At base
.85 percent.....	2	At base
Sealright		
.17 percent.....	No leakage ^a
.475 percent.....	3	At seam on base
.85 percent.....	2	At seam on base

^aThese containers did not leak during the six-day period of storage.

In a test made with containers filled with cultured and with sweet milk, the expansion of the sides, as expressed in thirty-seconds of an inch, was as follows:

	After storage at 40° F. for 7 days		After storage at 80° F. for 1 day	
	Diameter 1	Diameter 2	Diameter 1	Diameter 2
Sweet milk.....	4	1	2	2
Cultured milk.....	6	4	6	4

More bulging occurred when the bottle was filled with cultured milk. Also when the bottles were kept at room temperature, cultured milk caused them to leak, whereas sweet milk did not. When leakage occurred, it was usually evident at the seams and along the base. Canco containers were used in these tests.

Another experiment was run to see if the lactic acid in the cultured milk was responsible for the leakage. Samples of each of the three types of paper bottles were filled with water containing lactic acid in three different strengths. The results showed that the greater the amount of lactic acid in the water, the sooner the container leaked (Table 22).

Melting point of paraffin. To find out to what extent bulging was affected by the melting point of the paraffin used to waterproof the container, an experiment was conducted with paraffins of eight different melting points. Twelve containers were treated with each kind of paraffin at both 170° and 190° F. The containers were filled with milk which was preserved with formaldehyde while stored at 72° F. for three days. Measurements of the two sides of the containers were taken every 24 hours.

No very significant differences in bulging occurred among the containers coated with the different paraffins, possibly with the exception of the paraffin having the lowest melting point in the National Wax

TABLE 23.—BULGING OF PAPER CONTAINERS AS AFFECTED BY THE KIND OF PARAFFIN USED FOR COATING
(Averages of 12 containers stored at 72° F. for three days; the milk was preserved with formaldehyde)

Kind of paraffin	Melting point of paraffin	Tempera- ture of applying paraffin	Expansion of container in 32d's of an inch	
			Diam. 1	Diam. 2
National Wax.....	120-122	° F. 170	8	10.8
		190	8	12.7
	123-125	170	8	9.9
		190	8	10.8
	128-130	170	8	10.0
		190	8	13.0
	133-135	170	8	10.3
		190	8	10.8
Socony Vacuum.....	125-127	170	8	10.0
		190	8	11.4
	133-135	170	8	9.9
		190	8	11.3
Gulf Refining.....	125	170	8	10.25
		190	8	11.7
	135	170	7.4	11.7
		190	7.0	11.5

series (Table 23). It may be that more significant differences would have been obtained if the containers had been stored at a higher temperature. Even when the containers were stored at 70° F., bulging was shown to be affected by the temperature at which the paraffin was applied, more expansion occurring on the sides extending to the stapled top (at Diameter 2) in the containers treated at 190° F. than in those treated at 170° F. The greater rigidity of the containers paraffined at the lower temperature is probably caused by the additional paraffin retained on the paper.

Rapidity of Heat Transfer

A container that allows heat to be transferred rapidly to the milk is undesirable from a practical standpoint. For this reason experiments were performed to find out how the rate of temperature change in

TABLE 24.—TEMPERATURE CHANGES OF WATER STORED IN PAPER BOTTLES AND IN GLASS BOTTLES

Time stored	Temperature of storage when reading was taken	Temperature of water in glass bottles	Temperature of water in paper bottles			
			Light-weight with high melting point paraffin	Light-weight with low melting point paraffin	Heavy-weight with high melting point paraffin	Heavy-weight with low melting point paraffin
<i>minutes</i>	° F.	° F.	° F.	° F.	° F.	° F.
0.....	63.5	44.6	44.6	44.6	44.6	44.6
30.....	64.1	49.1	49.1	48.2	47.8	47.4
60.....	64.6	51.4	50.4	51.4	50.4	51.4
90.....	65.1	54.0	52.7	53.8	52.7	53.8
120.....	65.5	55.7	54.6	55.4	54.5	55.6
150.....	65.4	57.9	55.6	56.9	55.4	57.2
180.....	66.0	58.8	57.5	57.8	57.2	58.8
210.....	66.4	59.9	58.1	59.0	57.7	59.6
240.....	67.0	61.2	59.6	60.2	59.2	60.8
270.....	66.2	62.6	60.8	61.2	61.2	62.6
300.....	65.9	64.4	62.6	63.5	62.6	63.8

either milk or water put in paper containers compared with that put in glass bottles.

In the first experiment heavy- and light-weight Pure-Pak containers treated with both paraffins of a high and a low melting point were compared with quart glass bottles. All containers were filled with water at 44.6° F. and held encased at 63.5° to 67° F. for periods varying from nothing to 300 minutes. At the end of each 30-minute period the contents of a bottle from each set was thoroly mixed and its temperature recorded.

The temperature changes that occurred in the stored water during the different periods of storage, while slightly less in the paper containers than in the glass bottles, did not vary enough to be of any practical significance (Table 24).

A further study was made of the relative differences in temperature changes that may occur in the contents of paper and glass containers. This time milk was used and the containers were placed in regulation cases, wooden for the glass bottles and fiber for the paper bottles. Several cases of each kind were placed on tables at room temperature and also at approximately 100° F. Each case held 12

TABLE 25.—TEMPERATURE CHANGES OF MILK STORED IN PAPER BOTTLES AND IN GLASS BOTTLES PACKED IN REGULATION CASES
(Average of all containers in case)

Time stored	Temperature of storage when reading was taken	Temperature of milk in—	
		Paper bottles	Glass bottles
Stored at room temperature			
<i>hours</i>	° F.	° F.	° F.
0.....	72.50	42.80	42.80
1.....	72.50	46.76	48.56
2.....	72.50	47.30	50.90
3.....	72.68	48.20	54.68
5.....	71.60	51.62	56.48
7.....	71.60	53.60	58.64
Stored at about 100° F.			
0.....	96.00	34.00	34.00
1.5.....	102.00	38.30	57.30
4.0.....	100.00	50.40	82.20
6.0.....	108.00	59.80	87.50

containers. The milk was thoroly mixed in each bottle and the temperature recorded.

The temperature change was much faster in the cased glass bottles than in the cased paper containers (Table 25). When allowed to stand at room temperature for only three hours, the milk in the glass bottles had reached a slightly higher temperature than that reached in the paper containers after seven hours. The differences in temperature between the paper and glass containers was even greater when the cases were stored at 100° F.

Possible Dilution of Milk by Foam Remover

Foaming may present a problem in filling the quart-size paper container, especially when the milk is homogenized. Foaming results from agitating the milk and it is particularly pronounced when the temperature is below 40° F.^{11*}

To avoid losing milk and soiling the outside of the container, a device for blowing down the foam before closing the container has

been developed by the manufacturers of the Pure-Pak filling machine. Tho either steam or air is used in the foam remover, steam is considered somewhat more effective and certainly it is more desirable from a sanitary point of view. A possible drawback to steam is that it may cause too great dilution of the milk. The steam is passed thru a trap, just before it is released, and the milk is exposed to the jet of steam for 7/6 second.

To measure the amount of dilution that might be caused by the jet of steam, tests were made. The bottom of aluminum dishes was covered with calcium chlorid. The dishes were then brought to constant weight by placing them in a 100° C. oven for 24 hours, after which they were placed in a desiccator and stored in a room at 40° F. To collect the steam, the cold dishes were placed under the defoamer for 7/6 second and then weighed.

The amount of steam collected on five dishes varied from .249 gram to .3832 gram and averaged .3073 gram. Assuming that approximately the same amount of steam would be taken up by the milk as by the aluminum dishes, the dilution per quart of milk would be .0323 percent, an infinitesimal amount.

Protection Against Off-Flavor Caused by Sunlight

One of the major problems in marketing bottled milk is to protect it against sunlight. Hammer and Cordes^{5*} first reported on the deleterious effect of sunlight upon the flavor of milk. Frazier^{4*} explained the action of light as a catalyst in the oxidation of the butterfat. Tracy^{13*} later showed that the common burnt flavor resulting from the action of sunlight upon milk was an effect not upon the butterfat but upon the serum constituents. Tracy and Ramsey^{14*} found that the exposure of washed cottage cheese curd packed in glass to the direct or indirect rays of the sun would cause the characteristic burnt flavor to occur after 15 minutes. In a study of homogenized milk, Tracy^{13*} found that bottled homogenized milk exposed to sunlight will acquire the burnt flavor more rapidly than will regular milk.

To determine to what extent light rays are able to penetrate the container and affect the flavor of milk, heavy- and light-weight Pure-Pak containers and glass bottles filled with both regular and homogenized milk were exposed to the direct sunlight. A duplicate set of containers used as controls was not exposed to the sunlight. After a period of 15, 60, and 90 minutes a set of the containers put in the sunlight was removed and placed in a refrigerator at 40° F. All samples were then judged according to the official collegiate score card; this gives flavor a perfect score of 25.^a

^aAfter these tests were made the official collegiate score card was changed. Flavor is now given a perfect score of 45.

TABLE 26.—EFFECT OF SUNLIGHT ON FLAVOR OF MILK STORED IN PAPER BOTTLES AND IN GLASS BOTTLES

(Official collegiate score card, with a perfect score of 25,^a was used; all six control samples not exposed to sunlight scored 22.5)

Kind of container	Kind of milk	Score after 15 minutes' exposure	Score after 60 minutes' exposure	Score after 120 minutes' exposure
Heavy-weight paper.....	Homogenized.....	21.00	20.25	19.75
	Regular.....	21.50	21.00	20.50
Light-weight paper.....	Homogenized.....	20.50	19.75	19.25
	Regular.....	21.25	20.75	20.00
Glass.....	Homogenized.....	20.25	18.00	17.50
	Regular.....	21.00	18.50	18.00

^aAfter these tests were made, the official collegiate score card was changed. Flavor is now given a perfect score of 45.

Because of a burnt flavor, all samples exposed to the sunlight scored below the control samples (Table 26). The milk in the heavy paper containers was least affected by the sunlight; the milk in the glass bottles was most affected. Homogenized milk acquires the burnt flavor more readily than regular milk under the same conditions.

Protection of Ascorbic-Acid Content of Milk

It having been shown that paper protects milk flavor against the effect of sunshine to a greater extent than does glass, several experiments were performed in which milk in both glass containers and in paper containers was exposed to sunshine for different lengths of time and the resulting effect upon flavor and ascorbic acid (vitamin C) determined. Representative results are given in Table 27.

To determine to what extent paper may protect the vitamin-C content of milk exposed to indirect sunlight, as when a bottle is left on the kitchen table, a comparison was made of the ascorbic-acid content

TABLE 27.—EFFECT OF SUNLIGHT ON THE FLAVOR AND ASCORBIC-ACID CONTENT OF MILK IN PAPER BOTTLES AND IN GLASS BOTTLES EXPOSED FOR DIFFERENT PERIODS

Time exposed to sunlight	Intensity ^a of sunshine flavor when milk was stored in—		Ascorbic acid per liter when milk was stored in—	
	Paper bottles	Glass bottles	Paper bottles	Glass bottles
			mg.	mg.
0.....	0	0	18.30	18.30
.5.....	0	1.5	16.90	8.15
1.0.....	1	2.5	14.65	7.55
2.0.....	2	4.0	13.40	2.24
4.0.....	2	5.0	7.14	1.02

^aThis flavor score was arbitrarily established. Zero indicates the absence of the burnt flavor; 5 indicates the most intense burnt flavor.

of milk in paper and glass bottles before and after standing for two hours on a laboratory table exposed to light rays that entered the room thru frosted panes. The milk contained 18.8 milligrams of ascorbic acid per liter at the beginning of the experiment, and after two hours' exposure to the indirect light rays at room temperature that in the glass bottles contained 15.91 mg. per liter and that in the paper containers contained 18.56 mg. per liter.

The results of these two experiments clearly show that paper milk containers protect the ascorbic acid (vitamin C) in milk against the oxidizing action of either direct or indirect sunlight much better than do glass bottles. Even tho milk is not looked upon as one of the chief sources of vitamin C, the retention of the full amount of this vitamin in the milk is of some importance from a nutritional standpoint. Protection, to be effective, must be supplied both before and after the milk reaches the kitchen.

Ability to Withstand Pressure

In practical use a milk container must be capable of withstanding a certain amount of rough handling. To find out if the paper container satisfies this requirement, an experiment was performed to measure the amount of pressure that had to be applied to a paper container filled with water before it collapsed enough to leak. The machine illustrated in Fig. 9 was used to measure the pressure. Tests were made on 24 paper containers of each of the three types. Half of the containers of all three types were placed upright. The rest of the Pure-Pak and Canco containers were laid on their sides, and the Sealright containers were placed bottom up.

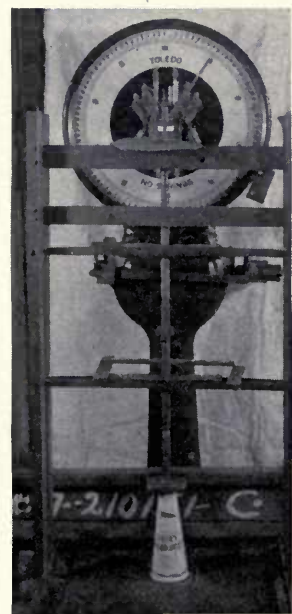


Fig. 9.—Apparatus used to study ability of paper containers to withstand pressure.

The amount of pressure needed to cause leakage varied with the type of container and the way in which the pressure was applied (Table 28). One of the paper containers was able to withstand up to 325 pounds of pressure, and all the bottles withstood up to 80 pounds. This amount of resistance to pressure makes it possible for the paper containers to stand up under the amount of handling necessary in delivering them.

TABLE 28.—PRESSURE NECESSARY TO PRODUCE LEAKAGE IN PURE-PAK, CANCO, AND SEALRIGHT CONTAINERS
(Expressed in pounds)

Test No.	Pressure needed on Pure-Pak container ^a when placed—			Pressure needed on Canco container ^b when placed—		Pressure needed on Sealright container ^c when placed—	
	Up-right	On side with stapled top	On side with tucked-in top	Up-right	On side	Up-right	Bottom up
1.....	90	90	...	105	125	205	220
2.....	90	90	...	108	125	245	210
3.....	90	95	...	98	110	205	212
4.....	85	90	...	103	125	245	220
5.....	82	100	...	103	125	205	190
6.....	88	87	...	100	120	200	172
7.....	92	..	315	95	120	180	198
8.....	80	..	320	95	115	180	197
9.....	85	..	320	94	118	230	180
10.....	85	..	325	115	130	200	150
11.....	85	..	320	104	125	150	170
12.....	90	..	320	102	122	170	165
Average.....	87	92	320	102	122	209.5	199.9

^aLeakage occurred first at corners at top or bottom. ^bLeakage occurred first at seam on top of bottle. ^cLeakage occurred first along seam at top and bottom.

Consumer Reaction to Paper Milk Containers

After the Pure-Pak paper container had been used on the University milk route for several weeks, a questionnaire was sent to each of the 300 customers in an attempt to secure the housewives' opinions of the merits of the single-service paper container (Table 29). Space was provided on the questionnaire for comments, favorable or unfavorable,

TABLE 29.—OPINIONS OF 300 CUSTOMERS CONCERNING RELATIVE MERITS OF PURE-PAK AND GLASS BOTTLES

Question	Number of times answer was—		
	Paper bottle	Glass bottle	No difference
1. Which do you think is more sanitary?.....	125	33	63
2. In which does milk have the best flavor?.....	10	37	172
3. In which does milk keep better?.....	16	31	172
4. In which does milk freeze faster in winter?.....	23	37	160
5. In which does milk warm up the faster?.....	34	33	152
6. Which bottle wastes the least space in the refrigerator?.....	156	16	50
7. Which is easier to pour from?.....	61	117	43
8. With which is it easier to separate cream from milk?.....	11	145	63
9. Which is easier to pack for traveling, picnics, etc.?.....	131	34	56
10. Which is more convenient for housewife?.....	96	71	54
11. If sold for the same price, which would you prefer when—			
a. Delivered to home?.....	102	97	22
b. Purchased at store?.....	110	78	33
12. If sold for 1 cent less per quart in paper, which would you prefer when—			
a. Delivered to home?.....	147	60	14
b. Purchased at store?.....	140	55	26

that the customers wished to make. A summary of these comments follows:

Favorable

1. No washing of bottles.
2. No breaking or chipping of bottles.
3. No bottle to return.
4. Moisture does not condense as much as on glass.
5. Empty containers make garbage containers.
6. Good containers for other foods in ice box.
7. Containers easy to dispose of.
8. Good for kindling.
9. More easily handled.
10. Easier to close after being opened.
11. Less weight on refrigerator shelf.
12. Bottles easy to open.
13. Paper construction eliminates noise of bottles and cases in early morning deliveries.

Unfavorable

1. Difficult to see cream line.
2. Paraffin comes off in milk.
3. Difficult to see amount left in container.
4. Container leaked milk.
5. Difficult to close container.
6. Lacks rigidity.
7. Container tips easily.
8. No bottle to put note in for delivery man.
9. Bottle becomes soft after sitting around awhile.
10. Wax comes off on refrigerator shelf.
11. Harder to wash off before putting away.
12. Hot milk cannot be returned to container.
13. Difficult to dispose of.
14. When placed against freezing coil, milk freezes.
15. Container melts if left on stove while cooking.
16. Dogs carry away or puncture with teeth after delivery to porch.

Consumer reaction to the Canco container was studied in a similar way. Just before sending out this second questionnaire, the customers were served with milk in glass containers for a few days. Results of the 136 questionnaires returned are summarized in Table 30.

Again the customers were invited to make comment for or against the use of paper milk containers. A summary of these comments follows:

Favorable

1. I hope the paper bottle is here to stay.
2. Container takes up less space in refrigerator.
3. No danger of chipped glass. Paper container must be handled a little more carefully but worth the extra effort.
4. Paper container eliminates bottle washing and handling.
5. We did not like the paper container at all at first but we have learned to like it as it is more sanitary and more convenient in every way.
6. Great improvement over glass bottle.

TABLE 30.—OPINIONS OF 136 CUSTOMERS CONCERNING RELATIVE MERITS OF CANCO AND GLASS BOTTLES

Question	Number of times answer was—		
	Paper bottle	Glass bottle	No difference
1. Which do you think is more sanitary?.....	104	13	19
2. Which conserves more space in refrigerator?.....	120	6	10
3. In which can cream be more easily separated?.....	16	74	46*
4. Which is easier to pour from?.....	81	36	19
5. Which is more convenient?.....	107	21	8
6. Your preference at same price per quart when—			
a. Delivered to home?.....	100	22	14
b. Purchased at store?.....	60	30	46
7. Your preference if 1 cent cheaper in glass when—			
a. Delivered to home?.....	40	57	39
b. Purchased at store?.....	29	48	59
8. Your preference if 1 cent cheaper in paper when—			
a. Delivered to home?.....	95	8	33
b. Purchased at store?.....	64	12	58

*Many of these customers use homogenized milk.

7. We particularly like paper containers for the space they save.

8. I carried a paper bottle of milk 200 miles and it arrived in perfect condition.

9. More convenient to close and put back in the refrigerator than glass bottle because it will not spill if upset.

10. I like this container very much.

Unfavorable

1. You cannot tell how much milk you have without lifting out the container and looking into it.

2. I like the glass bottle better chiefly because I can see the cream and get it easier.

3. The flecks of paraffin in the milk are quite objectionable.

4. The paper container is more difficult to pour from, as one cannot see as well as when pouring from glass.

5. There is usually a little milk left in the container.

6. Milk tends to gush out when container is nearly emptied.

7. Paper container gets soft if warmed.

8. Milk will not keep sweet as long in paper. Leaks thru if kept in paper too long. Has a slight taste of the paper.

9. The disposal of the container is more trouble than washing a glass bottle.

After the Canco container had been used consistently for several weeks the Pure-Pak container was again used alternately with the Canco and the glass bottle. Change in design of one of the paper containers made it desirable to obtain the consumers' comments on this change. On this questionnaire was included the question, "Do you favor the use of paper milk containers?" One hundred and fifty-nine answered *yes* and 38 answered *no*. In answer to the question, "Has your opinion of the desirability of the single-service paper milk container changed since we first started using them?" 51 answered *yes* and 123 answered *no*. This was as expected, for it had been observed

that a number of the customers who first objected to paper containers became enthusiastic about them later, whereas no person once satisfied with them reported a change of preference to the glass bottle.

The third type of container used for University milk was the Seal-right. After customers were served milk in this container for about three weeks they were asked which factors are likely to have greatest influence upon the consumers' acceptance of paper milk containers. Fifty-eight stated convenience features, such as ease of handling, opening, closing; 77 mentioned the single-service feature; 16 indicated the way in which the container packed in the refrigerator; 59 thought the sanitary feature important; 16 included danger of glass breakage; and the same number thought cost would be a contributing factor.

Approximately sixteen months after the paper container was introduced on the University milk route and after all three types had been tried, the glass bottle was used for a period of about two weeks and then another questionnaire was sent out. To the question, "Do you favor the use of the paper container?" 136 replied *yes*, 14 replied *no*, and 5 did not reply. It was interesting that after using paper containers for a period of sixteen months, only 9 percent of the users objected to them; whereas on the first questionnaire put out about a month after paper bottles were first used, about 44 percent stated their preference for glass. Apparently a good many housewives changed their opinions after using paper containers for some time. This accounts for the fact that 33 percent of the housewives on the last questionnaire stated that altho they did not at first prefer paper bottles to glass, they had come to like them better than glass.

On this questionnaire the customers were again asked to state what they considered the advantages and disadvantages of paper milk containers. Following is a summary of the answers:

Advantages	Number of times men- tioned	Disadvantages	Number of times men- tioned
1. Less space.....	105	1. Cannot see cream line.....	32
2. No washing.....	97	2. Paraffin cracks off.....	12
3. More sanitary.....	59	3. Lack of transparency.....	10
4. No bottle to put out.....	50	4. Does not pour well.....	9
5. Easier to handle.....	31	5. Leakage.....	9
6. No disadvantage.....	29	6. Not easily disposed of.....	8
7. No breakage.....	29	7. Not easy to handle.....	6
8. Light weight.....	29	8. Carried off by dogs.....	3
9. Pours well.....	21	9. Slippery.....	1
10. Better for picnic use.....	11		
11. Easier to open.....	10		
12. Splendid kindling.....	8		
13. Convenient.....	5		
14. Good for garbage.....	5		
15. Noiseless.....	4		
16. Does not chip box.....	1		

Four years after the paper containers were first used by the University Creamery a final questionnaire was sent to each customer. The Canco container was in use at the time. A summary of the results follows:

1. Did you like paper containers when you started using them? Yes, 174; No, 47.
2. Do you like paper containers now? Yes, 219; No, 12.
3. If you had your choice would you choose paper or glass containers when sold—
 - a. At the same price? Paper, 212; glass, 12.
 - b. At 1 cent premium in paper? Paper, 115; glass, 79.
 - c. At 1 cent premium in glass? Paper, 212; glass, 12.

The final results were even more favorable for paper containers than the results of earlier surveys. Ninety-five percent of those who answered the questionnaire indicated a preference for paper containers. Fifty percent of the housewives who returned the questionnaire stated they would pay a premium of 1 cent a quart in order to get their milk in paper containers. These results are particularly significant in light of the recent shift to store selling, for which paper milk containers are especially well adapted. It is likely, therefore, that since the paper bottle has been favorably received by consumers, its acceptance by the fluid-milk industry will depend on whether milk can be marketed in it as cheaply as in the glass bottle.

From the replies to the four questionnaires it is evident that consumers have decided preferences for certain features in a paper milk container. A bottle is wanted that is easily opened and closed, has the pouring lip well protected, permits separation of the milk and cream, is easily handled and sturdy enough to be handled without undue care. In general a square container is preferred.

Adaptability of Paper Container to Plant Operations

During the five years that the paper milk containers were in use, the following observations were made as to their general adaptability for plant operations compared with glass bottles.

Advantages

1. They make less noise and confusion in the plant.
2. Less water gets on the floor and there is less moisture in the air during bottling.
3. Bottling is less hazardous.
4. There is less difficulty in maintaining proper temperatures in the milk storage rooms during bottling.
5. The bottled milk takes up less space in the refrigerated rooms.
6. Delivery trucks are easier to load, and the loads are less than half as heavy for the same volume of milk.
7. Time is saved in delivery of the milk.
8. There is less rise in temperature of the milk during delivery.

Disadvantages

1. The milk that drops on the outside of the container during the filling process is difficult to remove satisfactorily.
2. A sliver of paraffin sometimes appears in the milk.
3. In some cases the foaming of milk (particularly homogenized) makes it difficult to seal the package without getting foam on the outside of the container.
4. No satisfactory method has been developed for detecting containers that are not properly filled.
5. The paper boxes in which the containers are packed for delivery to milk customers cannot be kept clean and attractive after being used a few times.
6. An occasional container will leak.

SUMMARY AND CONCLUSIONS

To answer two fundamental questions about paper milk containers—are they sanitary and are they practical for the fluid-milk trade—an investigation was undertaken by the University of Illinois. Bacteriological studies were made of the various processes involved in making pulp and paper, fabricating the containers, and paraffining and filling them. Studies were also made to determine the bactericidal action of paraffining. In investigating the practicability of the containers tests were made of such features as their strength, resistance to bulging and leakage, ability to keep heat from being transmitted to the contents, the degree of protection afforded against the harmful effect of sunlight on the flavor of milk, and the adaptability of the containers to plant operations. Surveys were also made to get consumer reaction to the paper bottles. Three types of containers, represented by the Pure-Pak, Canco, and Sealright bottles, were studied.

In the manufacture of paper there are three regular operations which make it possible to produce sanitary paper without resorting to any special bactericidal treatment, according to surveys in two pulp and paper mills. Cooking the wood to free the pulp fibers and bleaching the pulp completely destroy all microbial life. The third operation, drying the paper by passing it over the hot drier rolls, kills all bacteria except those in the spore stage. This operation has a bactericidal effect comparable to that of a thoro sterilizing treatment given utensils and glass bottles in the milk plants.

The paper from which containers are made has as a rule some bacterial spores, the number depending on the kind of pulp and mill operations practiced. Paper can be made which has but a few bacteria, as was shown by examining 170 samples of paperboard. Almost 60 percent of the samples had a count of less than 100 bacteria per gram, and the average count was 120.

It was found that when paper was inoculated with a bacterial suspension and then allowed to dry at room temperature, the inoculating organisms gradually died. Thus paper undergoes self-purification when it is stored in rooms where it dries.

The finished paper milk containers found on the market at the time this study was made were in excellent sanitary condition. This was shown by a bacteriological examination of 2,607 quart containers. The paper from which these containers were made had been manufactured at different mills and the containers had been fabricated at several carton factories and sent out to different milk plants. The Standard Method for examining glass bottles or more exacting modifications of it were used to test the paper containers. The examination showed that 878 of the paper containers (about 33 percent) were sterile and those not completely free from bacteria contained as a rule only a few. The test for coliform bacteria was made on a large number of the containers and negative results were obtained.

Pure-Pak containers were not contaminated to any extent during the processes involved in finishing their fabrication at the milk plant. The amount of adhesive used for sealing the seams in the bottom of a container showed an average bacterial count of only 2. The hot melted paraffin remained sterile during the paraffining operations for over 2,000 containers. Most of the containers passing thru the cooling unit were not contaminated by a single bacterium, as shown by calculations from the tests in which opened petri dishes containing nutrient agar were exposed to the air in the cooling unit. Even after an hour of exposure the average number of colonies on the plates was only 7.6.

Experiments with three types of paper containers and with glass bottles showed that when the pouring lip of a container is not well covered it contaminates the milk as it is poured out. Containers closed with only a cap or a disk fitting inside the opening are not sanitary.

Bacteria do not penetrate the walls or seams of paper containers. After the containers had been submerged in a bacterial suspension for as long as 48 hours, none of the bacteria from the suspension were found in the milk.

The machines used for filling the Pure-Pak, Canco, and Sealright containers did not contaminate the milk beyond what usually occurs in filling glass bottles. They do not present any special problems in the matter of washing and sterilizing.

To test the bactericidal action of paraffining, experiments were made with small paper plaques in the laboratory and with regular quart-size containers paraffined in the Pure-Pak machine in the milk plant. When the paper plaques were heavily inoculated by submerging them in a bacterial suspension less than one-tenth of 1 percent of the organisms survived when the paraffining was done at 170° F. for about 20 seconds.

In the experiments conducted at the milk plant 3,653 containers were paraffined and then examined for bacteria. When the containers were heavily contaminated, only an occasional container showed the

presence of the inoculating organism after paraffining. This was true when the paraffining was done at different temperatures. In one experiment 1,200 containers were handled in the usual way except that the operator heavily contaminated his hands by dipping them in a bacterial suspension. The containers were paraffined for about 24 seconds at 170°, 180°, and 190° F. All these containers were free from the inoculating organisms except one paraffined at 190° F.

Paper, as a rule, contains some bacteria, mostly in the spore stage, in which they cannot be destroyed by the heat of the paraffin. The practically sterile condition of the containers indicates that paraffining has further bactericidal action than that caused by heat. Apparently the paraffin imprisons most of the spore-forming organisms and keeps them from getting into the milk.

The amount of paraffin adhering to paper containers depends upon the temperature of application, more paraffin being retained at lower temperatures. When paraffin with a melting point of 125° to 127° F. was used it did not begin to melt until it reached approximately 120° F. When the melting point of the paraffin was 135° to 137° F., a temperature of approximately 131° F. was needed before it began to melt.

In the process of filling Pure-Pak paper containers with milk, the dilution from the steam foam remover is only approximately .03 percent and therefore has no noticeable effect on the milk.

The amount of water absorbed by filled paraffined containers varies primarily with the time and temperature at which they are stored. Slightly more moisture is absorbed when a container is made from heavy paper. The amount of bulging that takes place in the stored containers increases with an increase in temperature.

When containers are not packed in cases, the temperature of the milk in paper bottles changes a little more slowly than that in glass bottles. When the containers are packed in cases which are completely filled, the rate of temperature change is much slower in the paper bottles.

The amount of pressure needed to cause a paper container to leak varies with the type of container and the position it is in when the pressure is applied. All the paper bottles tested stood as much as 80 pounds of pressure before they leaked.

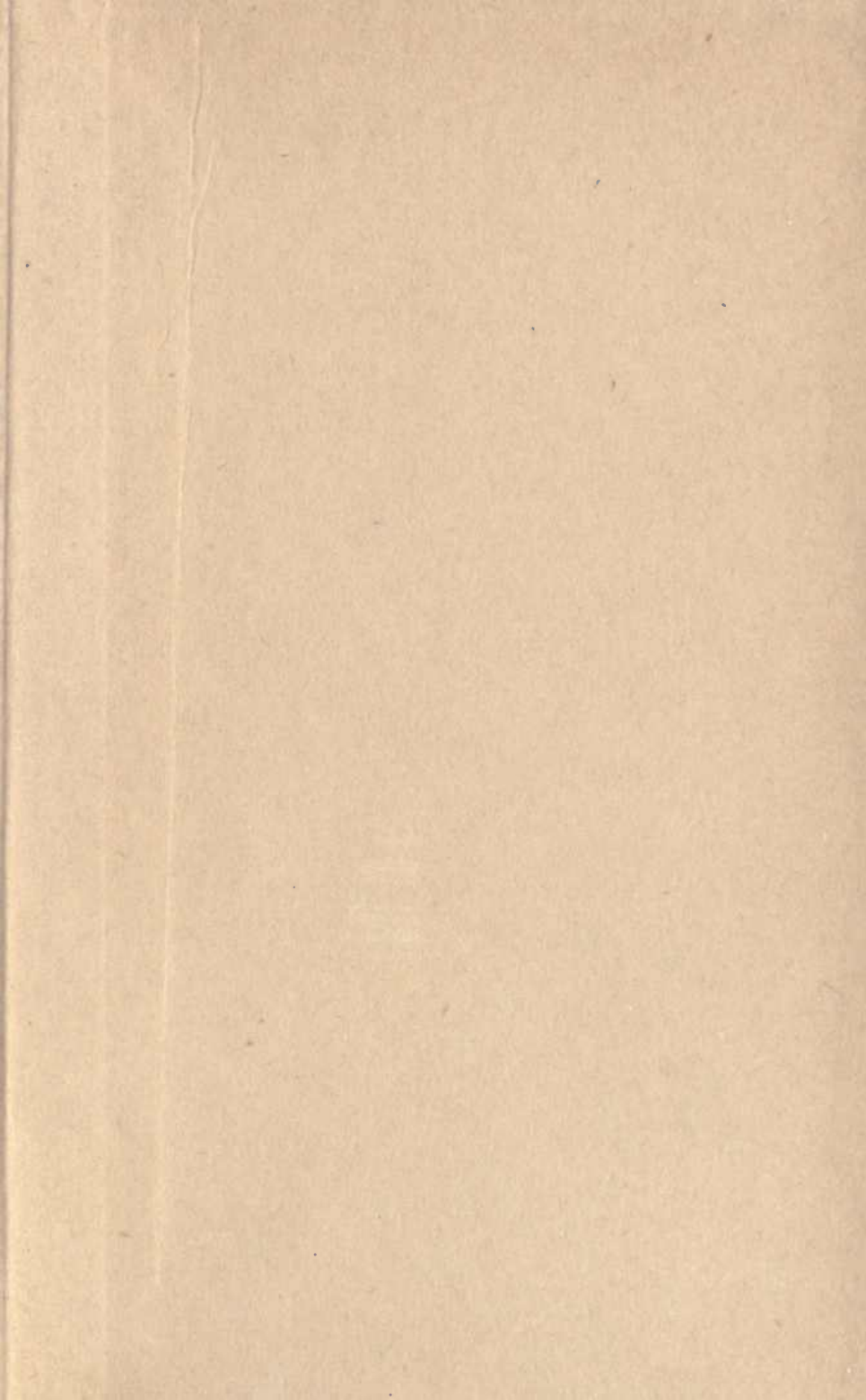
The ascorbic-acid content of milk is reduced and the milk takes on a burnt taste when it is stored in either glass or paper containers exposed to the sunlight, but the change takes place less rapidly in the paper bottle.

In the opinion of consumers, as well as by actual plant tests, milk containers made from paper are practical. After four years of using glass and paper bottles at intervals, 95 percent of the consumers who returned questionnaires stated that they preferred the paper bottle.

The results of these investigations clearly show that paper milk containers are sanitary as well as practical for fluid-milk distribution.

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