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Influence of added iodine on the bacterial flora of milk

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INTRODUCTION.

Pasteurization of milk has been the subject of much controversy and criticism since Soxhlet in 1886 proposed that milk could be treated with heat to preserve it and lessen its chance of disseminating pathogenic bacteria. The arguments against the use of pasteurized milk have been numerous. A few of the more salient adverse criticisms included:

1. Exposure to the heat used in pasteurization alters the nutritional value of the milk, particularly the vitamin content.
2. Pasteurization involves the use of expensive equipment, resulting in a higher cost of milk production.
3. Pasteurization does not protect the milk against re-contamination after the completion of the pasteurization process.
4. The consumption of pasteurized milk over an extended period of time tends to establish a putrefactive intestinal flora in the individual consuming the pasteurized milk.

The pasteurization of milk, when properly performed, materially reduces the total bacterial count of the milk, eliminates the spread of pathogenic bacteria through the agency of milk, and improves the keeping quality of the milk. A suitable substitute for the pasteurization process would have to produce, as a minimum effect, results comparable with those obtained by pasteurization, and should eliminate some of the objectionable features.

A chemical method offers a possible substitute if we are to preserve milk without the use of heat. The chemical group of elements known as the halogens have long been used as disinfectants, because of the strong bactericidal properties possessed by members of the group. Iodine and certain of its compounds are very strong bactericidal agents. Iodine has also been used both for therapeutic and prophylactic purposes. Therefore, the addition of iodine to milk has been suggested for its combined therapeutic and prophylactic value, and for the control of bacteria in milk.

The purpose of this study was to determine the amounts of different iodine compounds required to inhibit the growth of bacteria commonly found in milk.

LITERATURE REVIEWED.

Rideal[16] stated that iodine as an oxidizing agent is more feeble than the related halogens, chlorine and bromine, but that it combines more readily, in dilute solutions, with the protoplasm of organisms, thereby poisoning them. Davaine and Marchal de Calvin[8] were the first to introduce iodine as an antiseptic. The former found that seven milligrams of iodine killed _Bacillus anthracis_ in 1000 milligrams of liquid. Griffiths[9] stated that one part in 10,000 destroyed _Sarcina lutea_ in half an hour.

The iodide ion is more toxic than the bromide or chloride ions. Meyerhof[14] found that iodine in concentrations of 0.05 N reduced the respiration of nitrifying bacteria by 44 per cent, and in concentrations of 0.1 N, by 57 per cent.

According to Buchanan and Fulmer[3], iodine has been used in the emergency sterilization of water. This is usually followed by treatment with sodium thiosulphate to remove the excess of iodine. It is efficient when 3.8 to 5.0 parts per million of iodine are present.

Ishikawa[10] concluded that potassium iodide and potassium iodate, even in low concentrations, tended to inhibit ammonia formation by proteolytic bacteria and by urea-splitting bacteria. No stimulation was evidenced. Potassium iodide has no demonstrable selective inhibiting action, different bacteria being affected almost equally by the presence of the iodide.

Suiffet[26] studied the time required for sixteen different iodine-bearing preparations to exert antiseptic action on "colon bacilli", "streptococci", and "pyocyaneous bacilli". Iodized water seemed to be the most active, but as slow chemical changes affect its antiseptic value, the potassium iodide-iodine solution seemed preferable.

Sutton and Krauss[27] stated that the presence of a small amount of iodine in milk increased the rate of bacterial growth, probably through an increase in the rate of metabolic processes. Approximately 0.0001 gms. of iodine per 100 ml. of milk seemed most favorable. Bacterial growth was retarded in milk containing 0.004 gms. of iodine per 100 ml.

Devereux[7] claimed that the preliminary results with the direct iodizing of milk indicated that colloidal iodine readily combines with milk proteins and unsaturated fatty acids. The brown discoloration disappears from the milk within twenty to thirty seconds after the addition of enough iodine to make a concentration of 100 parts per million. Even at this concentration the taste of the milk is not altered, nor does abnormal flavor develop as the milk ages and sours.

Scharrer and Schwaibold[18] claimed that the iodine normally found in milk is only partly in the form of inorganic compounds. Mostly it is bound to the organic substances of the serum, which is free from protein, fat, or lactose; a smaller and variable quantity is in combination with protein; while the fat either contains no iodine, or contains it in such small traces as to be of no significance.

Magee and Glennie[12] stated that heat may cause the disappearance of 20 per cent of the total iodine of separated milk. About 83 per cent of the total iodine of separated milk is in the diffusible form. The ratio of diffusible to non-diffusible iodine is the same in the milk after it has been heated as before.

EXPERIMENTAL.

Media Employed.

The majority of the samples of milk used in this study were obtained from the Department of Dairy Industry of the Massachusetts State College. Occasionally milk was obtained from samples which were accepted at the bacteriological laboratory for routine examination. The whole milk used in the preliminary studies consisted of raw milk samples obtained from the college supply. All milk, to which iodine was to be added, was placed in eight-ounce dilution bottles in 100 ml. quantities. Rubber stoppers with a cotton-plugged air vent were used. The sample bottles for the raw milk were sterilized before they received the milk, to avoid possible laboratory contamination.

The milk used for the pure culture studies was handled in the same manner as was the raw milk. One-hundred ml. quantities of the milk were placed in eight-ounce dilution bottles and sterilized in the autoclave for 15 minutes at 15 pounds steam pressure. By this procedure, sterile milk was obtained which varied only slightly in consistency and color from natural untreated milk. The milk showed a slight tendency to curdle, and occasionally a batch would tend to be a brownish-white in color. A pronounced cooked odor was present in all sterilized samples.

The nutrient agar and the nutrient broth used were prepared according to the Standard Methods of Milk Analysis[20]. The nutrient agar for plating was placed in 12 to 15 ml. quantities in test tubes and sterilized.

The nutrient broth for parallel studies with the milk was placed in 100 ml. quantities in eight-ounce dilution bottles and sterilized in the autoclave for 20 minutes at 15 pounds steam pressure.

All plating of Streptococcus lactis was done with lactose agar, rather than with nutrient agar, since colonies were much more definite and easier to count on the lactose agar. The composition of the lactose agar was:

Beef Extract	5 gms.
Bacto Peptone	5 gms.
Bacto Lactose	10 gms.
Bacto Agar	15 gms.
Water	1000 ml.

All dilutions in the study were made in either 9.0 ml. or 99.0 ml. quantities of sterile distilled water.

Iodine Employed.

The halogens exert their disinfectant action only in the free state. In combination they may modify the properties of the compound and make it possibly more antiseptic, but they cannot be disinfectant in the same sense as is the halogen element in the free state. The above fact had to be considered when iodine compounds were chosen for the work at hand. Iodine was employed in three forms for the study: tincture of iodine, aqueous iodine-potassium iodide solution, and a commercial iodine preparation "Iodine Suspensoid". The free iodine content of all compounds used was carefully checked according to the accepted methods employed in volumetric analysis.

Tincture of iodine was prepared fresh at least weekly in 50 ml. amounts, according to the following formula:

2.5 grams KI in 2.5 ml. water
3.5 grams I_2
Alcohol to make 50 ml.

Tincture prepared according to the U. S. P. theoretically contains 0.007 grams of free iodine per 0.1 ml. The iodine content of the samples prepared deviated but very little from the theoretical. The minimum free iodine content determined in a sample was 0.0068 grams, and the maximum was 0.0072 grams in 0.1 ml.

An aqueous solution of iodine (Gram's Iodine, which is a modified Lugol's solution) was prepared in 30 ml. quantities according to the following formula:

0.2 grams KI
0.1 grams I_2
30 ml. distilled water

This preparation was found to be very unstable and had to be prepared fresh daily if comparable results were to be obtained. The theoretical amount of free iodine in the aqueous solution was 0.0033 grams per 1.0 ml. The results of analysis of the samples prepared showed that .0030 grams per 1.0 cc. was the minimum free iodine content determined and the maximum was 0.0033 grams.

A commercial iodine preparation, "Iodine Suspensoid, Merck", was obtained through the courtesy of Merck & Co., Inc. The Suspensoid, according to Chandler[6], is a so-called "colloidal iodine". The Suspensoid is produced by the acidification at 0° C. of a sodium iodo-hypoidite solution containing acacia as a protective colloid. It consists of an extremely fine suspension of iodine capable of forming, almost instantly, a saturated aqueous solution of iodine, which does not vary in strength as long as free iodine remains in suspension. The preparation contains 20 per cent free iodine.

Considerable difficulty was experienced at first in handling the iodine suspensoid. It is a heavy opaque liquid which very tenaciously clings to the side of the burette, making it impossible to make a correct reading. The final method determined on was to add the liquid, drop by drop, from a calibrated burette. The same burette was used throughout the study. An analysis of a considerable number of drops indicated that no material variations in the free iodine content of separate drops was being obtained. The Suspensoid contained, according to analysis, 0.0072 grams of free iodine per drop.

Cultures Employed.

No attempt was made, in the pure culture work, to study the effect of iodine on every possible contaminant of milk. An attempt was made to take representative organisms of possible groups of contaminants encountered in the everyday handling of milk. The following organisms were used in the study:

1. *Escherichia coli*
2. *Aerobacter aerogenes*
3. *Bacillus subtilis*
4. *Streptococcus lactis*
5. *Staphylococcus aureus*
6. *Eberthella typhi*

All cultures employed, except that of Streptococcus lactis, were standard stock cultures which were carried in the laboratory.

The culture of Streptococcus lactis used was isolated from moderately soured milk. Excellent results were obtained by carrying the culture on tomato agar, making a transfer each day. All cultures used were checked according to Bergey's "Manual of Determinative Bacteriology"(2).

Technique Employed.

The preliminary work in the study was done on raw milk. One quart of milk was designated as a single sample and was proportioned into 100 ml. quantities as has been explained under "Media Employed". Each sample was thoroughly mixed before being divided into 100 ml. quantities, in order that each portion would contain approximately the same number of organisms. Tincture of iodine was used as the source of free iodine for the preliminary work. Nine 100 ml. portions usually constituted a single sample and the samples were analyzed on that basis. One portion was always maintained as a control, and to the remainder of the portions tincture of iodine was added. The iodine was added using 0.1 ml. quantities as a basis for varying the concentration of iodine in each portion. The aim was to vary the concentration of iodine in the sample in order to find the minimum concentration necessary to sterilize the sample. Thus, an individual sample of milk would be set up in the following manner:

Portion No.	1	2	3	4	5	6	7	8	9
Quantity of Iodine in ml.	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8

After allowing a given period of time (contact period) to elapse, each portion was plated in order to obtain The bacteria count; the control portion was usually plated in dilutions of 1-100 and 1-1,000. The portions to which iodine had been added were plated in dilutions of either 1-100 or 1-10 depending upon the concentration of iodine present in the sample. All original dilutions from the milk were made using 1.0 ml. transfers. Each dilution was plated in duplicate in order to have a check on the results. The plating was done with nutrient agar. Incubation was at 37.5° C. for 48 hours. At the end of the incubation the plates were counted using a Buck Colony Counter. All studies in the work were done on a quantitative basis.

Studies were made using pure cultures of organisms representative of the organisms commonly found in milk. Much difficulty was encountered at first in controlling the numbers of organisms placed in the milk. It was advisable to inoculate the milk in such manner that the number of bacteria present would correspond approximately with the number of bacteria found in a moderately contaminated sample of milk. An attempt was made to keep the total bacteria count of each sample within 100,000 per cubic centimeter. All inoculations were made from eighteen-hour broth cultures of the organism, except in the case of the spore-forming <u>Bacillus subtilis</u>. Samples containing free spores were prepared from seventy-two-hour broth cultures of *Bacillus subtilis*. The approximate count per ml. was determined in eighteen-hour broth cultures of all organisms used in the study. Thus, by the use of dilutions, each portion of milk could be inoculated with approximately the number of organisms desired. After inoculation, the samples were incubated for from forty-five minutes to one hour before the addition of the iodine, to allow the organisms to recover from the shock of being transferred to a fresh medium. Parallel studies were made in nutrient broth in order to determine to what extent the high protein content of the milk interfered with the bactericidal properties of the iodine. Tincture of iodine, modified Lugol's solution, and "Iodine Suspensoid" were used as sources of free iodine in the pure culture work.

The technique of running the samples was similar to the technique employed in the raw milk work. Iodine was added in varying concentrations and the concentration necessary to sterilize the samples was determined. Different periods of contact were investigated, ranging from five minutes to twenty-four hours in length.

RESULTS.

The bacteria counts obtained in the study are presented in tables 1 to 16, inclusive. Each table shows the original count of the sample, the contact period used, the form of the iodine used, and the bacteria count in the presence of varied quantities of iodine. Representative samples for each organism used were tabulated and are presented in table 17, to illustrate the effect of varied contact periods on the various organisms. There were a few discrepancies in the results with individual samples: in some cases no plate was made with the low concentrations of iodine used, in other cases a portion may have been accidentally spoiled, or the plate made proved to be contaminated. In all cases where no count was obtained, the fact is indicated in the table by the use of a dash (-).

An attempt was made to evaluate the results on a basis which could be compared with the standard milk counts of the Massachusetts Milk Regulation Board(15) for market milk. The allowable count for Grade A milk - Raw is 100,000 bacteria per ml., and for Special Milk - Raw is 50,000 bacteria per ml. An arbitrary value of 5,000 bacteria per ml. was taken as being representative of a good grade of Certified Milk - Raw. The results obtained did not parallel the standard counts in every instance; therefore strict comparisons cannot be made in all cases.

Although the author believes that the conditions of the study compare favorably with those normally encountered in handling market milk, the fact must be remembered that conditions beyond control may arise in connection with any given sample.

The preliminary work with raw milk, shown in tables 1 and 2, yielded results which proved to correlate very closely with those obtained in the pure culture work. A summary of the preliminary work can be made. The figure given under the column headed "Gms. I.", in the table following, represents the minimum concentration of iodine found necessary to sterilize 100 ml. quantities of milk, with an initial bacteria count as stated.

Control Count per ml.	Contact Period	Quantity of Tinc. of I.	Gms. I.
Up to 100,000	4 hrs.	0.7 ml.	0.049
" " 50,000	" "	0.5 "	0.035
" " 5,000	" "	0.4 "	0.028

Table 1.

Bacteria Counts in Raw Milk with Varied Concentrations of Tincture of Iodine, 30 Minutes Contact.

Sample No.	Control	Concentrations of Tincture of Iodine in Ml.				
		0.2	0.3	0.4	0.5	0.6
R1	176,000	-	1,500	1,600	1,300	-
R2	21,600	16,200	500	200	0	0
R3	445,000	184,800	14,800	-	-	-
R4	114,000	45,200	4,700	3,800	-	-
R5	4,900	400	100	0	0	0
R6	3,950	700	90	0	0	0
R7	16,000	7,100	340	0	0	0
R8	25,800	-	1,300	600	200	0

0 = Plates were sterile.

- = Either no plating was done at this concentration, or plates were contaminated and spoiled. In most instances the portions containing the low concentrations of iodine were not plated unless it was expected that the concentration would be sufficient to kill the organisms present.

Table 2.

Bacteria Counts in Raw Milk with Varied Concentrations of Tincture of Iodine.

Time	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.				
			0.3	0.4	0.5	0.6	0.7
2 1/2 hours contact	R9	34,000	-	20	10	0	0
	R10	47,000	-	30	20	0	0
	R11	61,000	-	50	30	0	0
	R12	73,000	-	-	140	50	0
	R13	111,000	-	-	5,400	760	310
4 hours contact	R9	Counts same as above.	-	0	0	0	0
	R10		-	60	0	0	0
	R11		-	40	0	0	0
	R12		-	-	150	0	0
	R13		-	-	5,700	560	0

Escherichia coli was chosen for the study because it is a representative organism of the non-sporulating group of possible contaminants of milk, and is a very good index of fecal contamination. The results shown in tables 3, 4, and 5, from the study of Esch. coli, using tincture of iodine, modified Lugol's solution, and "Iodine Suspensoid" as sources of iodine, are summarized in the following chart.

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 50,000	30 minutes	Tinc. of iodine	0.6 ml.	0.042
	" " 5,000	" "	" " "	0.5 "	0.035
	" " 50,000	" "	Iodine Suspensoid	6 drops	0.0432
	" " 5,000	" "	" "	5 "	0.0360
	" " 50,000	" "	Lugol's solution	12.0 ml.	0.0399
	" " 5,000	" "	" "	10.0 "	0.0333
In 100 ml. of Broth	" " 5,000	" "	Tinc. of iodine	0.3 "	0.021
	" " 5,000	" "	Iodine Suspensoid	1 drop	0.0072
	" " 50,000	" "	Lugol's solution	5.0 ml.	0.0166
	" " 5,000	" "	" "	3.0 "	0.0099

Table 3.

Bacteria Counts of Esch. coli Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.					
			0.1	0.2	0.3	0.4	0.5	0.6
In Milk	1	6,500		650	530	100	0	0
	2	650		80	50	0		
	3	1,450		790	415	45	0	0
	4	7,750		7,100	6,000	1,200	0	0
	5	3,200		1,500	185	10	0	0
	6	54,700		-	2,350	1,060	400	0
	7	9,700		240	130	5	0	
In Broth	N1	3,600	850	250	0			
	N2	3,000	1,050	30	0			
	N3	4,800	700	20	0			
	N4	1,300	600	0	0			

Table 4.

Bacteria Counts of Esch. coli Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Iodine in Drops *					
			1	2	3	4	5	6
In Milk	8	7,750		720	-	150	0	0
	9	23,200		-	1,230	1,050	300	0
	10	1,470		-	790	0	0	0
	11	49,700		1,360	1,200	780	400	0
	12	4,200		1,200	830	170	0	0
In Broth	N5	3,000	0	0	0			
	N6	4,900	0	0	0			
	N7	14,300	800	0	0			
	N8	6,700	0	0	0			

* Iodine Concentration of Drop = 0.0072 gms.

Table 5.

Bacteria Counts of Esch. coli Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Lugol's Solution Present; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Iodine in Ml.											
			1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
In Milk	13	9,000						8,400	4,200	290	0	0	0	0
	14	1,560						650	210	0	0	0	0	0
	15	10,000						-	-	1,300	120	0	0	0
	16	18,000						-	-	-	2,900	1,200	280	0
	17	53,000						-	-	-	-	4,000	1,900	0
In Broth	N9	13,800	6,600	900	0	0	0							
	N10	18,000	9,200	2,210	970	280	0							
	N11	45,300	12,800	6,000	1,400	740	0							
	N12	4,200	970	210	0	0	0							

Aerobacter aerogenes was chosen for the study because it is a representative organism of the non-sporulating, non-fecal group of contaminants. The particular strain of Aer. aerogenes used (Mass. Agric. Exp. Sta. Strain A101) is a heavily encapsulated organism and produces a very viscous ropy milk if allowed to grow unchecked in milk. The results, shown in tables 6 and 7, from the study of Aer. aerogenes, using tincture of iodine and "Iodine Suspensoid", are summarized as follows:

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 50,000	30 minutes	Tinc. of iodine	0.6 ml.	0.042
	" " 5,000	" "	" " "	0.5 "	0.035
	" " 50,000	" "	Iodine Suspensoid	6 drops	0.0432
	" " 5,000	" "	" "	5 drops	0.0360
In 100 ml. of Broth	" " 5,000	" "	Tinc. of iodine	0.1 ml.	0.0070
	" " 50,000	" "	Iodine Suspensoid	3 drops	0.0216
	" " 5,000	" "	" "	1 drop	0.0076

Table 6.

Bacteria Counts of Aero. aerogenes Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine Present; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.					
			0.1	0.2	0.3	0.4	0.5	0.6
In Milk	18	1,030			930	10	0	0
	19	7,100			1,500	350	0	0
	20	22,700			11,450	1,200	670	0
	21	58,000		7,200	6,000	2,600	900	0
	22	3,800			1,440	210	0	0
In Broth	N13	1,050	0	0	0			
	N14	1,160	0	0	0			
	N15	6,670	10	0	0			
	N16	4,000	0	0	0			

Table 7.

Bacteria Counts of Aero. aerogenes Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid Present; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Iodine in Drops *					
			1	2	3	4	5	6
In Milk	23	11,030		9,000	1,300	500	40	0
	24	2,700		1,410	630	210	0	0
	25	4,200		-	900	450	0	0
	26	8,000		2,700	1,600	200	0	0
	27	68,300		32,400	10,600	1,200	300	0
In Broth	N17	1,050	0	0	0			
	N18	1,160	0	0	0			
	N19	1,670	5	0	0			
	N20	44,000	1,700	60	0			

* Iodine Concentration of Drop = 0.0072 gms.

Bacillus subtilis was chosen for the study because it is a spore-forming organism and is representative of the aerobic sporulating contaminants which might be encountered in milk. The results, shown in tables 8 and 9, from the study of B. subtilis can be summarized as follows:

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 5,000	4 hours	Tinc. of iodine	0.4 ml.	0.028
	" " 2,500	" "	" " "	0.3 "	0.021
	" " 2,500	" "	Iodine Suspensoid	3 drops	0.0216
In 100 ml. of Broth	" " 2,500	" "	Tinc. of iodine	0.1 ml.	0.007
	" " 2,500	" "	Iodine Suspensoid	1 drop	0.0072

Table 8.

Bacteria Counts of B. subtilis Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine Present; 4 Hours Contact.

	Sample No.	Control	Concentrations of Iodine in Ml.			
			0.1	0.2	0.3	0.4
In Milk	28	1,510		240	20	0
	29	760		110	0	0
	30	1,950		420	0	0
	31	5,300		1,100	70	0
	32	2,500		140	0	0
	33	850		80	0	0
In Broth	N21	1,500	0	0	0	
	N22	770	0	0	0	
	N23	2,060	0	0	0	
	N24	1,030	0	0	0	
	N25	2,150	0	0	0	
	N26	810	0	0	0	

Table 9.

Bacteria Counts of B. subtilis Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid Present; 4 Hours Contact.

	Sample No.	Control	Concentrations of Iodine in Drops*			
			1	2	3	4
In Milk	34	1,510	-	170	0	0
	35	1,950	-	140	0	0
	36	1,300	-	100	0	0
	37	2,550	-	635	0	0
	38	850	-	300	0	0
In Broth	N27	1,500	0	0	0	
	N28	760	0	0	0	
	N29	2,060	0	0	0	
	N30	1,030	0	0	0	
	N31	2,150	0	0	0	
	N32	1,810	0	0	0	

* Iodine Concentration of Drop = 0.0072 gms.

Streptococcus lactis was chosen for the study because it is the index organism of the acid producing group of bacteria which are commonly found in normal milk. The results obtained using Strep. lactis in pure culture, as shown in tables 10 and 11, are summarized in the following chart:

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 50,000	4 hours	Tinc. of iodine	0.6 ml.	0.042
	" " 5,000	" "	" " "	0.4 "	0.028
	" " 50,000	" "	Iodine Suspensoid	6 drops	0.0432
	" " 5,000	" "	" "	4 "	0.0364
In 100 ml. of Broth	" " 50,000	30 minutes	Tinc. of iodine	0.4 ml.	0.028
	" " 5,000	" "	" " "	0.2 "	0.014
	" " 20,000	" "	Iodine Suspensoid	3 drops	0.0216
	" " 5,000	" "	" "	2 "	0.0144

Table 10.

Bacteria Counts of Strep. lactis Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine Present; 4 Hours Contact in Milk; 30 Minutes Contact in Broth.

	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.					
			0.1	0.2	0.3	0.4	0.5	0.6
In Milk	39 *	1,010	-	940	600	0	0	0
	40 *	1,120	-	920	640	0	0	0
	41	6,900	-	-	1,620	1,190	0	0
	42	65,200	-	-	21,300	3,100	520	0
	43	11,600	-	-	3,400	1,300	0	0
In Broth	N33	1,200	170	0	0	0		
	N34	1,050	200	0	0	0		
	N35	17,000	3,100	510	0	0		
	N36	64,000	-	7,800	420	0		

* 2 hours contact.

Table 11.

Bacteria Counts of Strep. lactis Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid Present; 4 Hours Contact in Milk; 30 Minutes Contact in Broth.

	Sample No.	Control	Concentrations of Iodine in Drops *					
			1	2	3	4	5	6
In Milk	44	1,130	-	-	710	60	0	0
	45	47,000	-	-	3,070	1,340	900	0
	46	5,400	-	-	2,600	1,030	0	0
	47	11,800	-	-	4,800	3,600	230	0
In Broth	N37	1,200	940	0	0	0		
	N38	3,020	1,200	0	0	0		
	N39	16,300	3,400	420	0	0		
	N40	1,070	780	0	0	0		

* Iodine Concentration of Drop = 0.0072 gms.

Staphylococcus aureus was chosen for the study because it is a pathogenic organism of the suppurative type and is frequently used as an index organism of that group. The results obtained using Staph. aureus in pure culture, as shown in tables 12, 13, and 14, are summarized in the following chart:

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 100,000	1 hour	Tinc. of iodine	0.7 ml.	0.049
	" " 5,000	" "	" " "	0.6 "	0.042
	" " 5,000	" "	Iodine Suspensoid	6 drops	0.0432
	" " 50,000	" "	Lugol's solution	11.0 ml.	0.040
In 100 ml. of Broth	" " 5,000	" "	Tinc. of iodine	0.2 ml.	0.0140
	" " 5,000	" "	Iodine Suspensoid	2 drops	0.0144
	" " 50,000	" "	Lugol's solution	5.0 ml.	0.0166
	" " 5,000	" "	" "	4.0 "	0.0153

Table 12.

Bacteria Counts of Staph. aureus Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine Present; 60 Minutes Contact.

	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.						
			0.1	0.2	0.3	0.4	0.5	0.6	0.7
In Milk	48	3,050	2,200	1,010	480	100	45	0	0
	49	1,950	-	-	540	10	20	0	0
	50	1,300	-	-	600	80	0	0	0
	51 *	16,800	-	-	-	5,100	1,240	700	0
	52	200,000	-	-	-	-	2,300	160	0
In Broth	N41	3,150	390	0	0				
	N42	1,300	910	0	0				
	N43	6,800	820	0	0				

* 30 Minutes Contact

Table 13.

Bacteria Counts of Staph. aureus Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid Present; 60 Minutes Contact.

	Sample No.	Control	Concentrations of Iodine in Drops *						
			1	2	3	4	5	6	7
In Milk	53	3,030		2,170	1,160	450	60	0	0
	54	1,900		-	720	40	0	0	0
	55	1,310		-	640	70	0	0	0
	56	17,800		-	-	7,100	1,350	700	0
	57	5,200		-	1,300	830	110	0	0
In Broth	N44	5,150	690	0	0	0			
	N45	13,500	1,400	350	0	0			
	N46	6,900	750	0	0	0			
	N47	8,000	960	0	0	0			

* Iodine Concentration of Drop = 0.0072 gm.

Table 14.

Bacteria Counts of Staph. aureus Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Lugol's Solution Present; 60 Minutes Contact.

	Sample No.	Control	Concentrations of Iodine in Ml.								
			4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
In Milk	58	30,000					130	0	0	0	0
	59	49,000					1,100	310	0	0	0
	60	62,000					-	2,300	450	70	0
	61	36,000					-	600	40	0	0
In Broth	N48	27,000	270	0	0						
	N49	44,000	430	0	0						
	N50	64,000	1,300	0	0						
	N51	34,000	620	0	0						

Eberthella typhi was chosen for the study because it is a pathogenic organism of the intestinal type and is an adequate index organism of that group. The results obtained using *Eberthella typhi*, as shown in tables 15 and 16, are summarized in the following chart:

	Control Count per ml.	Contact Period	Type of Iodine	Quantity of I.	Grams I.
In 100 ml. of Milk	Up to 25,000	30 minutes	Tinc. of iodine	0.6 ml.	0.042
	" " 5,000	" "	" " "	0.3 "	0.021
	" " 25,000	" "	Iodine Suspensoid	6 drops	0.0432
	" " 5,000	" "	" "	4 "	0.0288
In 100 ml. of Broth	" " 5,000	" "	Tinc. of iodine	0.3 ml.	0.021
	" " 5,000	" "	Iodine Suspensoid	4 drops	0.0288

Table 15.

Bacteria Counts of E. typhi Inoculated into Sterile Milk and into Nutrient Broth, with Varied Concentrations of Tincture of Iodine; 30 Minutes Contact.

	Sample No.	Control	Concentrations of Tincture of Iodine in Ml.					
			0.1	0.2	0.3	0.4	0.5	0.6
In Milk	62	8,500	-	4,100	110	0	0	0
	63	6,000	-	2,100	0	0	0	0
	64	5,850	-	2,450	0	0	0	0
	65	7,800	-	-	50	0	0	0
	66	5,900	-	3,500	0	0	0	0
	67	3,500	-	2,280	0	0	0	0
	68	34,600	-	13,200	-	4,200	1,000	0
In Broth	N52	6,950	3,500	1,100	0	0		
	N53	4,600	-	1,130	0	0		
	N54	4,400	-	1,050	0	0		
	N55	7,500	-	-	0	0		

Table 16.

Bacteria Counts of E. typhi Inoculated into Sterile Milk and Into Nutrient Broth, with Varied Concentrations of Iodine Suspensoid; 30 Minutes Contact.

	Sample No.	Control	Concentration of Iodine in Drops *					
			1	2	3	4	5	6
In Milk	69	8,500				210	0	0
	70	6,000		900	0	0	0	0
	71	25,800		11,600	2,500	1,300	300	0
	72	7,800		-	0	0	0	0
	73	4,100		800	0	0	0	0
	74	15,900		3,400	2,700	350	0	0
	75	3,500		-	70	0	0	0
	76	4,600		1,180	230	0		
In Broth	N56	6,900	3,400	2,100	1,170	0	0	0
	N57	4,600	-	1,700	540	0	0	0
	N58	7,500	-	-	2,100	100	0	0

* Iodine Concentration of Drop = 0.0072 gm.

Table 17 shows the effect of the period of contact with iodine on bacteria. The summarized results indicate the optimum period of contact for destroying each organism, so no further summary is necessary here. One further item must be mentioned, however. It appears that a concentration of iodine not sufficient to sterilize the sample regardless of the length of its period of contact, will effectively reduce the bacteria count. Referring to table 17, the sample containing Strep. lactis shows an 83 per cent reduction of bacteria in the presence of 0.4 ml. of tincture of iodine, whereas 0.5 ml. sterilized the same sample after 3 hours contact. In the presence of 0.4 ml. of the tincture of iodine the 83 per cent reduction of bacteria was obtained after 60 minutes contact. From this point no significant change was observed in the count up to 4 hours. No bacteria counts were made for contact periods greater than four hours. These results indicate that a point was reached, after sixty minutes contact, where all of the available iodine ions had apparently been utilized in the destruction of 83 per cent of the organisms. Similar results were apparent with all of the pure cultures employed in the study.

The criticism might be made that the bacteria counts used in the study were for the most part representative of a relatively good grade of milk. However, in the opinion of the author, a bacteria count in excess of 100,000 per ml. is at the present time the exception rather than the rule with market milk, if the records of the Service Bacteriologist of the Massachusetts Agricultural Experiment Station may be considered as representative.

Table 17.

Bacteria Counts Illustrating the Effect of Varied Periods of Contact

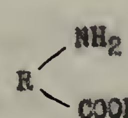
in the Presence of Tincture of Iodine.

Organism	Concentrations of Iodine in ml.	Control	Contact time in hours.									
			1/12	1/4	1/2	3/4	1	1 1/4	1 1/2	2	3	4
Escherichia coli	.4	1,020	0	0	0	0	0					
	.5	5,600	0	0	0	0	0					
Aerobacter aerogenes	# (.4	2,300	170	160	120	40	70	–	–	–	90	110
	(.5	2,300	50	0	0	0	0	–	–	–	0	0
Bacillus subtilis	* .3	1,450	1,430	1,380	1,240	–	890	–	450	220	60	0
	.3	1,500	0	0	0	0	0					
Streptococcus lactis	# (.4	6,900	–	–	1,720	–	1,100	–	–	1,170	1,220	1,190
	(.5	6,900	–	–	1,510	–	740	–	–	420	200	0
	.4	1,180	480	150	140	130	190	130	60	0	0	0
Staphylococcus aureus	# (.6	200,000	4,200	1,400	900	280	250	–	–	260	260	370
	(.7	200,000	3,000	600	150	90	0	–	–	0	0	0
Eberthella typhi	# (.4	3,500	530	0	0	0	0					
	(.5	3,500	0	0	0	0	0					

* = 72-hour culture (spores present).
# = Varied concentrations of iodine in two portions of the same sample.

DISCUSSION.

Three iodine preparations, tincture of iodine, modified Lugol's solution, and "Iodine Suspensoid", were used in an attempt to determine in what concentrations iodine would be bactericidal in milk. Comparative parallel studies were made using nutrient broth as a medium for growing the organisms. The results obtained in the study prove that iodine is bactericidal in nutrient broth and, to a lesser degree, is bactericidal in milk. The results obtained with nutrient broth are much more consistent than are those obtained with milk. In all cases the minimum concentration of iodine found necessary to destroy a given number of organisms in nutrient broth was lower than the minimum concentration necessary to destroy the same number of organisms in milk. Variations were found in the concentration of iodine necessary to sterilize different samples of milk with similar bacteria counts. In the author's opinion this variation of results can best be explained on the basis of the protein content of the media. Protein has a marked affinity for absorbing bactericidal and bacteriostatic agents such as iodine, chlorine, dyes, etc. The amino-acid composition of proteins and allied amphoteric substances furnishes groups with which these agents combine. If we think of the protein molecule as having the property of an amino acid, and write a type formula, as Loeb[11] does:



we have, in the language of Simon and Wood[19], receptors for either acidic or basic substances. A given quantity of protein may be expected to furnish receptors for only a limited quantity of iodine. In nutrient broth, allowing for experimental error, not much variation would be expected in the protein content of separate samples. However, the protein content of milk samples is apt to vary over relatively wide ranges and, in general, the protein content of milk may be expected to be higher than that of nutrient broth. Thus, because of the higher and more varied protein content of milk, a higher and more varied absorption of iodine by the protein would be expected in milk than in broth. That part of the iodine which had been absorbed by the protein would not be available for contact with the bacteria present in the media.

Although the protein content of the milk is the most probable absorbent of the added iodine, there is the possibility that a portion of the iodine may enter into combination with any unsaturated fatty acids present. However, the proportion of unsaturated fatty acids present is normally very low, and only traces of the iodine may be expected to be absorbed by this group. Hence, the amount of iodine absorbed by the milk fat would, under normal conditions, be negligible.

Another observation was that the higher the original bacteria count of a sample, with both milk and broth, the greater was the concentration of iodine found necessary to sterilize the sample. In some cases a variation of the protein content of the milk might be responsible for the need of the increased concentration of iodine. However, in the author's opinion, enough samples were run over a wide enough range of bacteria counts, to discredit the above explanation. There is apparently a more direct factor responsible which involves the free iodine and the individual bacterial cell. This factor leads to a discussion of the mechanism by which bacterial cells are destroyed by iodine. A discussion of the bactericidal action of iodine must, at best, be highly theoretical. The author has been unable to find any material pertaining directly to the means by which iodine destroys bacterial cells. There are apparently conflicting ideas as to the mechanism of destruction.

In the opinion of the author, the bactericidal properties of iodine may be fundamentally comparable with the bacteriostatic action of dyes. Stearn and Stearn[21, 22, 23, 24, 25] have made an exhaustive study of the bacteriostatic action of dyes and have proposed a chemical theory in explanation of bacteriostasis. Benians[1], Burke and Barnes[4], and Churchman[5] have expounded a physical theory of bacteriostasis. Drawing from the material available from these authorities, the author makes the following suggestions as to the mechanism of the bactericidal action of iodine.

The possibilities are that the action may be either physical or chemical in nature. If the action is physical, then there are two possibilities: either the iodine may be physically absorbed by the individual bacterial cell, thereby poisoning the cell, or the iodine may form a coating over the surface of the cell. If the latter statement is true there are many possibilities, the most likely of which are: the iodine may cause plasmolysis of the cell by removing the moisture in the cell through the process of osmosis; the iodine coating may form an impenetrable wall through which soluble food is unable to pass, thus resulting in the starvation of the organism.

The chemical theory is that the bacterial cell possesses receptors which are capable of uniting with the iodine ion, resulting in conditions being established in the cell not suitable for bacterial life.

The author wishes to emphasize the fact that, although theories can be made, no definite conclusions have yet been arrived at as to the exact mechanism of the destruction of bacteria by chemical bactericidal agents.

Iodine is active as a bactericidal agent only in the free state. It is necessary to prepare solutions of iodine in combinations with other products, usually potassium iodide, as it is very difficult to put free iodine into solution. However, in a discussion of results, only the iodine in the free state is considered. All results have been summarized on the basis of the weight in grams of the free iodine present. A comparison of tincture of iodine, modified Lugol's iodine solution, and "Iodine Suspensoid" indicates that there is but very little difference in the bactericidal efficiency of the preparations in terms of available free iodine. The modified Lugol's solution (aqueous solution of iodine-iodine-potassium iodide) seems to be the most active of the three preparations as a bactericidal agent in milk and in broth. The aqueous iodine solution is very unstable, as slow chemical changes affect its composition, and it has to be made frequently (usually daily), if a dependable product is desired for use.

When the Lugol's solution is added to milk quite a large dilution factor is introduced. If the Lugol's solution is compared with tincture of iodine and "Iodine Suspensoid" on the basis of the amount of free iodine per unit volume, we find that the Lugol's solution contains a much lower concentration of iodine per unit. Therefore, it is necessary to employ a greater number of units of the Lugol's solution to attain a concentration of iodine equivalent to the concentration obtained through the use of fewer units of either tincture of iodine or "Iodine Suspensoid". The Suspensoid results in the lowest dilution factor of the three preparations used.

The tincture of iodine and the "Iodine Suspensoid" are closely comparable on the basis of the free iodine content necessary to sterilize a given sample. The two products are only slightly less efficient bactericidal agents than is the aqueous iodine solution on the same basis. Tincture of iodine, although not permanently stable, is much more stable than is the aqueous solution of iodine. The "Iodine Suspensoid" is permanently stable as long as free iodine remains in suspension.

Iodine apparently had no marked selective action in its effect on bacteria. Heat pasteurization of milk destroys the vegetative cells but does not destroy spores. This does not seem to be true in the case of iodine added to milk. The spores of *Bacillus subtilis* required less iodine to destroy them than did many of the vegetative cells. It was interesting to note that the same concentrations of iodine necessary to destroy the vegetative cells (18-hr. culture) of *B. subtilis* would destroy the spores (72-hr. culture) if a longer contact period were used than was necessary to destroy the vegetative cells. The spores of *B. subtilis* were destroyed with a contact period of four hours, whereas the vegetative cells were destroyed almost instantly. *Staphylococcus aureus* proved to be the most resistant organism of those used in the study.

The author can make no definite statement as to whether or not the practical sterilization of market milk is possible by the use of added iodine. The fact must be remembered that, although iodine is an important element in human metabolism, excessive quantities of iodine are very toxic to humans. The present work has been solely a biological study and the author has no information relative to the iodine-tolerance of humans. Rather a large quantity of iodine is necessary to sterilize milk, and the author believes that iodization of market milk should not be practically undertaken without careful attention being given to the subject of iodine-tolerance of humans.

The practical iodization of milk presents further difficulties. The results obtained in this study show that the quantity of iodine necessary to sterilize milk is directly dependent upon the original bacteria count of the milk. Thus, it would be necessary to know the bacteria count of each batch of raw milk, in order that sufficient iodine could be added to sterilize the milk effectively.

The author believes that for certain purposes, such as for restricted hospital use, the practical sterilization of milk with iodine might be effected, provided that a consistently high quality milk is being obtained, with a bacteria count at least comparable with that of a good grade of Certified Milk. A high quality (low count) milk requires a lower concentration of iodine to effect sterilization than does milk with a higher count. The use of iodized milk should be under the supervision of a physician or some competent individual with a knowledge of the iodine-tolerance of the person consuming the milk.

In conclusion, the fact must be borne constantly in mind that whenever a chemical is used as a preservative in food there is an inherent possibility that difficulty may be encountered. Chemical treatment is frequently an inexpensive and sure method of preserving food. However, before a chemical can be accepted as a preservative, the fact must be proven that it is harmless to humans under every possible condition. Rosenau, in his "Preventive Medicine and Hygiene", states: "It may be stated as a general rule that any chemical which is poisonous in large amounts should be considered as poisonous in small amounts until the contrary is proven. In other words, the consumer is entitled to the benefit of the doubt." Extreme caution must be employed when the question of chemical preservation of food is considered. We must remember that if the use of one chemical is to be allowed, then the merits of other chemicals must be carefully weighed before their use can be refused. Thus the questions will be always before us: On what basis shall we permit the use of chemicals in food preservation, and where will the line of demarcation be set between a legitimate use of preservatives and of adulteration?

SUMMARY.

1. The effect of added iodine has been studied with reference to the bacterial flora of raw milk. Pure culture studies have also been made in sterilized raw milk and in nutrient broth. The organisms used in the pure culture work were: Escherichia coli, Aerobacter aerogenes, Bacillus subtilis, Streptococcus lactis, Staphylococcus aureus, and Eberthella typhi.

2. Iodine was used in the form of tincture of iodine, modified Lugol's iodine solution, and "Iodine Suspensoid" (Merck).

3. The modified Lugol's solution was slightly more efficient as a bactericidal agent in milk than was either the tincture of iodine or the "Iodine Suspensoid". The tincture of iodine and the "Iodine Suspensoid" are preferable because the preparations are more stable, and because they effect a lower dilution factor in milk than does the modified Lugol's solution.

4. The quantity of iodine necessary to effect complete sterilization of the milk was directly dependent upon the initial bacteria count of the milk: the higher the initial bacteria count of the sample, the higher was the concentration of iodine necessary to sterilize the sample.

5. Iodine apparently had no marked selective action for different bacterial species, in destroying the cells. The effects

of iodine upon vegetative cells and upon bacterial spores were comparable, except that a longer contact period with iodine was necessary to kill the bacterial spores.

6. The practical use of milk sterilized by iodization can not be recommended, except under the direction of persons familiar with the iodine-tolerance of the consumers.

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