Effect of Milk and Other Dairy Products on the Thermal Inactivation of Coxsackie Viruses

ALBERT S. KAPLAN, Ph.D., and JOSEPH L. MELNICK, Ph.D.

Evidence is presented of the ability of certain strains of Coxsackie virus to survive in cream in the temperatures currently set for that dairy product.

Perhaps the most widely occurring viruses in the human intestinal tract are those of the Coxsackie group.[1] The marked stability of these viruses has meant that when prevalent in the human population they can readily be recovered from human feces, sewage, and flies. Because fecal contamination of food and drink can occur in nature, the thermal destruction of Coxsackie viruses in the presence of water, milk, cream, and ice cream has been investigated.

Materials and Methods

Coxsackie Viruses from Human Sources—The stools of patients naturally infected with these viruses served as a source of these agents. Three different strains were employed in the heating experiments:

1. W–S, 1948 strain, in the form of pooled stools of poliomyelitis patients collected during an epidemic in Winston-Salem, N. C., in 1948. The stool specimens were pooled and frozen immediately after collection and were maintained at −20° C. Besides containing poliomyelitis virus, they were also found to contain a mixture of Coxsackie viruses belonging to two distinct antigenic types, B1 and A1.[2]

2. GJ strain, in the form of stools of a technician who had become ill with a laboratory infection. The virus isolated was identified as belonging to Type A4 (Texas–1).[3]

3. JLM strain, in the form of stools of an investigator who had also acquired an infection while working with these agents in the laboratory. In this instance the virus was found to belong to Type B1 (Connecticut–5).[3]

Mouse-Passaged Virus—In addition to human fecal material furnishing a source of virus, newborn mice infected with two strains were also used. These were selected because they represent agents which fall into two different antigenic and pathogenic groups.[3, 4] They were:

1. Tex.–1 strain (Type A4). The isolation and description of the properties of this strain have been given elsewhere.[3-6] The virus had been passed five times in newborn mice at the time of the present experiments.

2. Conn.–5 strain (Type B1). The isolation and properties of this strain have also been described.[3-6] The Conn.–5 strain had been passed nine times in mice before the heating experiments described were carried out.

Preparation of Viruses

Coxsackie Viruses from Human Sources—The procedure for the clarification and concentration of stools for the heat experiments has been described in detail.[7] In brief, 33 per cent sus-

pensions of fecal matter were prepared with distilled water and the virus concentrated 10-fold in an ultracentrifuge (100,000 × g for 60 minutes). The sediments which were thrown down in the ultracentrifuge were suspended in either water, milk, cream, or ice cream and were considered to be "undiluted" in the calculation of titration end points. All titrations were carried out by the inoculation of 0.02 ml. of each 10-fold serial dilution subcutaneously into newborn mice (24 hours old or less). The titer of these strains (calculated by the Reed and Muench method) is given in Table 1.

Mouse-Passaged Virus — Newborn mice were inoculated with seed virus of the Tex.–1 and Conn.–5 strains. Animals that came down with typical signs of disease were sacrificed; their heads, feet, tails, and skins were removed, and the animals then eviscerated. The carcasses remaining after this treatment were weighed and mixed in a chilled Waring blendor with nine volumes of each of the four media (water, milk, cream, and ice cream) mentioned previously. The resulting 10 per cent suspensions were spun lightly to sediment the tissue debris. Infectivity titrations were carried out on the supernates as above. For the respective titers and the amount of virus heated see Table 1.

Heating *—1. Holding method: The "standard" conditions for the pasteurization of milk as tested in these experiments were considered to be 61.7° C. (143° F.) for 30 minutes. Aliquots of 1 ml. of the stool and carcass suspensions were distributed into 2 ml. glass ampules which were then sealed and kept in a refrigerator or in an ice water bath until heated. When the ampules were to be heated, they were placed in weighted wire baskets, 10 cm. in length and 3.8 cm. in diameter; the mesh was 6 mm., sufficiently large to permit the free passage of water into the baskets. The latter were closed by means of a wire mesh lid to which a string was attached. The baskets with the enclosed ampules were then plunged into a constant temperature water bath, being held submerged at the desired temperature for 15 or 30 minutes. Immediately afterward they were withdrawn and dropped into an ice water bath. The temperature of the water bath was recorded by a thermometer graduated in 0.1° C. and it was held constant to within 0.05° C.

2. High-temperature short-time method (HTST): For this method of pasteurization, the "standard" conditions were considered to be 71.1° C. (160° F.) for 15 seconds. Experiments were carried out in thin, U-shaped glass capillaries with an average outside diameter of 1.71 mm. and with a length of 40 cm. (about 1.5 ml. in volume).[7] The capillaries were filled with the virus suspensions and both ends sealed. These capillaries were tied with cotton thread by means of which they were lowered into a water bath maintained at

Table 1—Infectivity Titer of Coxsackie Viruses Used in the Heating Experiments

Source of Virus	Strain	Titer (ID_{50})
Human stools	W–S 1948	$10^{-3.0}$
" "	JLM	$10^{-4.5}$
" "	GJ	$10^{-3.0}$
Murine muscle	Tex.–1	$10^{-7.5}$
" "	Conn.–5	$10^{-6.5}$

* The time and temperature relationships which obtain in official pasteurization equipment are in excess of the temperatures 61.7° C. (143° F.) for 30 minutes and 71.1° C. (160° F.) for 15 seconds as used in these experiments. For ice cream, pasteurization usually refers to a minimum of 68.3° C. (155° F.) for 30 minutes; a tentative standard of 79.4°C. (175° F.) for 25 seconds has also been recommended.

The authors are associated with the Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

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71.1° C.; after heating, they were promptly plunged into an ice water bath. The water bath in which HTST pasteurization was carried out was held to within 0.05° C. of the desired temperature.

Suspending media: Raw milk Grade B, was obtained from a local dairy. Pasteurized heavy cream (38 per cent fat) was purchased on the open market. The ice cream mix (12 per cent fat and 38 per cent total solids) was not aerated and had been pasteurized when received.

Phosphatase tests: In order to follow the extent of pasteurization, phosphatase tests were carried out on the heated suspensions by the method of Sanders.[13]

Determination of virus activity: After the virus suspensions had been heated, 0.1 ml. of a solution containing 2,000 units of penicillin and 10 mg. of streptomycin was added to each ml. of suspension. Newborn mice were then inoculated subcutaneously with 0.02 ml. amounts of the heated suspensions. At least two litters of mice—eight mice per litter—were inoculated with each sample, the animals being observed for a period of two weeks. Deaths which occurred during the first two days after inoculation were considered nonspecific, and those mice eaten by their mothers during the observation period were not considered in the final tabulations. If all or most of the animals in the litters died without paralysis having been observed, the test was repeated.

Experimental Results

Human Coxsackie Virus—1. Holding method: The results in the top part of Table 2 show that the viruses as present in the stool suspensions were inactivated by the holding method of pasteurization of milk (61.7° C. for 30 minutes). Inactivation of the W–S strain suspended in water, milk, or cream occurred after heating at 61.7° C., even though the specimens were submerged in the water bath for only 15 minutes, one-half the "standard" holding period. Suspended in ice cream, this strain was destroyed by heating at 65° C. for 15 minutes. The W–S strain suspended in water survived after being heated at 50° C. for 30 minutes, but was destroyed at 55° C. for 15 minutes. Milk protected the virus so that it was still infectious following heating at 55° C. for 30 minutes; however, it was destroyed at 61.7° C. for 15 minutes. Thus, with virus suspended in milk there is a margin of safety * of no more than 7° C. It is noteworthy that virus was destroyed at lower temperatures than milk phosphatase, the widely used criterion for milk pasteurization.

The W-S virus suspended in cream was inactivated when heated at 61.7° C. for 15 minutes. In the presence of ice cream, however, this virus was not destroyed by heating at 60° C. for 30 minutes, and it was necessary to increase the temperature to 65° C. before the virus was no longer detectable.

The JLM and GJ strains suspended in water and milk were inactivated by heating at 61.7° C. for 30 minutes. These strains suspended in cream and ice cream were inactivated by heating for 30 minutes at 62.7° C. and 71.1° C., respectively. Heating at other temperatures was not carried out.

2. High-temperature short-time method: The HTST method of pasteurization (71.1° C. for 15 seconds) was adequate for the inactivation of these strains of virus as present in human fecal matter. None of the three strains survived regardless of the type of suspending medium employed (see bottom part of Table 2). Inactivation occurred even when the holding time was only 7.5 seconds. Thus, by the

* By margin of safety, we mean the difference in degree centigrade between the standard prescribed minimum pasteurization temperature and the lowest point to which the temperature may be lowered and still cause thermal inactivation of virus.

Table 2—Thermal Inactivation of Human Coxsackie Virus

Strain	Temperature °C.	Temperature °F.	Time (Minutes)	Milk Phosphatase Test *	Fate of Newborn Mice Inoculated with Virus Heated in: Water	Milk	Cream	Ice Cream
W-S	65	149	15	ND	..	..	..	0/10 †
1948	61.7	143	15	36	0/15	0/8	0/8	..
	60	140	30	ND	..	..	..	12/14(12)
	60	140	15	ND	..	..	..	13/13(13)
	55	131	30	>1,000	0/8	10/10(10)	..	..
	55	131	15	>1,000	0/8	9/9(9)	..	..
	50	122	30	>1,000	6/6(2)	..	..	..
	50	122	15	>1,000	16/19(4)	..	..	..
JLM	71.1	160	30	ND	..	..	..	0/8
	62.7	145	30	ND	..	..	0/13	..
	61.7	143	30	6	0/8	0/16	..	..
GJ	71.1	160	30	ND	..	..	..	0/8
	62.7	145	30	ND	..	..	0/11	..
	61.7	143	30	6	0/16	0/8	..	..
				High-Temperature Short-Time				
			(Seconds)					
W-S	71.1	160	15	8	0/16	0/14	..	..
1948	71.1	160	7.5	>1,000	0/13	0/15	..	0/8
JLM	71.1	160	15	8	0/13	0/16	0/7	0/16
GJ	71.1	160	15	8	0/10	0/17	0/16	0/8

ND = Not done.

* The results of the phosphatase tests are expressed in mg. of phenol equivalent per 0.5 ml. of milk, following the method of Sanders.[8] According to this test, properly pasteurized milk should yield no more than 2 gamma of phenol equivalents per 0.5 ml. of sample.

† The numerator indicates the number of mice dead, the denominator the number of mice inoculated; figures in parentheses give the number of mice with observable paralysis.

HTST method of pasteurization, there was a minimum margin of safety by at least 7.5 seconds, considering 15 seconds to be the "standard" holding time.

Mouse-Passaged Virus—1. Holding method: Thermal inactivation of the Conn.-5 and Tex.-1 strains suspended either in water or in milk follows the same pattern found for the viruses as present in the stool suspensions, although the results with cream and ice cream were different. As the data in Table 3 indicate, virus suspended in water and heated at 50° C. for 30 minutes remains infectious for newborn mice. An increase of the temperature to 55° C. was sufficient to destroy this infectivity even though the specimens had been held in the water bath for only 10 minutes. Both strains suspended in milk resisted thermal inactivation at a temperature of 55° C., the specimen being held for 30 minutes in the water bath. However, virus in milk was destroyed by elevating the temperature to 61.7° C. with only 15 minutes of heating being necessary. If "standard" conditions for the holding method of pasteurization are considered to be 61.7° C. for 30 minutes, then for milk there is a margin of safety of less than 7° C.

The results obtained with cream with mouse-passaged virus were different from those obtained with human fecal virus. The Conn.-5 strain suspended

Table 3—Thermal Inactivation of Mouse-Passaged Coxsackie Virus

Strain	Temperature °C.	Temperature °F.	Time (Minutes)	Milk Phosphatase Test *	Fate of Newborn Mice Inoculated with Virus Heated in: Water	Milk	Cream	Ice Cream
Conn.–5	71.1	160	30	ND	..	..	..	0/13
	67.7	153.8	30	ND	..	..	0/11	0/14
	65.2	149.3	30	ND	..	..	0/15	0/16
	62.7	145	30	ND	..	..	5/12(1)	0/16
	61.7	143	30	6	0/8	0/8	..	5/14(4)
	61.7	143	15	36	0/21	0/27	..	..
	55	131	30	>1,000	0/14	3/15	14/14(7)	13/13(1)
	55	131	10	>1,000	0/8	7/7(1)	..	..
	50	122	30	>1,000	12/13(10)	10/10(3)	..	..
Tex.–1	72.1	161.8	30	ND	..	..	0/10	..
	71.1	160	30	ND	..	..	1/6(1)	0/16
	67.7	153.8	30	ND	..	..	9/12(8)	0/9
	65.2	149.3	30	ND	..	..	9/9(6)	2/9(1)
	62.7	145	30	ND	..	..	20/20(11)	..
	61.7	143	30	6	0/8	0/10	..	16/16(12)
	61.7	143	15	36	0/17	0/20	..	..
	55	131	30	>1,000	0/14	12/12(2)	..	..
	55	131	10	>1,000	0/15	13/13(0)	..	..
	50	122	30	>1,000	14/14(7)	9/9(4)	..	..
				High-Temperature Short-Time				
			(Seconds)					
Conn.–5	71.1	160	30	ND	..	..	0/15	..
	71.1	160	20	ND	..	..	2/15(2)	0/15
	71.1	160	15	8	0/15	0/14	5/16(5)	1/14(1)
	71.1	160	11	ND	0/16	0/11	..	..
Tex.–1	71.1	160	75	ND	..	..	1/15(1)	..
	71.1	160	60	ND	..	..	3/15(1)	..
	71.1	160	45	ND	..	..	9/14(8)	0/16
	71.1	160	30	ND	0/15	0/16	6/6(3)	0/10
	71.1	160	20	ND	0/16	5/15(5)	..	..
	71.1	160	15	8	0/21	3/13(2)	15/15(10)	0/14
	71.1	160	11	ND	0/13	5/10(4)	..	..

* Gamma phenol per 0.5 ml.

in cream survived heating at 62.7° C. for 30 minutes but was inactivated at 65.2° C. The Tex.–1 strain in the presence of this medium was still infectious after being heated for 30 minutes at 65.2° and 67.7° C. and even slightly so at 71.1° C.; however, at 72.1° C. virus failed to survive (see Table 3).

The Conn.–5 strain suspended in ice cream survived treatment at 61.7° C., but was destroyed at 62.7° C. for 30 minutes. The Tex.–1 strain remained infectious after being held for 30 minutes at 65.2° C., but was inactivated at 67.7° C.

The protective effect of milk, cream, and ice cream, brought out clearly in these experiments with mouse-passaged virus, is shown in Figure 1. The percentage of mice down with disease after being inoculated with virus suspended in these media is indicated for each temperature employed. Thus, Conn.–5 virus suspended in water and heated at 55° C. failed to infect any of the ani-

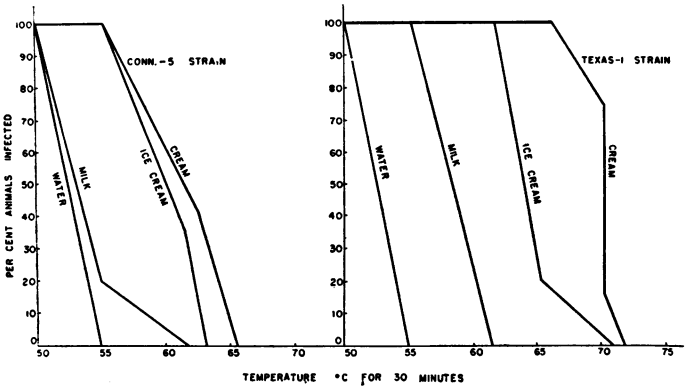

Figure 1—Protective effect of milk, ice cream, and cream on thermal inactivation of Connecticut-5 and Texas-1 Coxsackie viruses

mals, while virus suspended in milk and heated at that temperature caused infection of 20 per cent of the mice. Ice cream was even more protective; 36 per cent of the animals inoculated with virus heated at 61.7° C. came down with disease. Cream showed the greatest protective action by preventing destruction of virus at 62.7° C. to such an extent that 42 per cent of the animals showed signs of disease.

The capacity of dairy products to interfere with thermal inactivation of the virus is shown even more strikingly with the Tex.–1 strain. None of the animals inoculated with this strain suspended in water and heated at 55° C. came down, although all the mice were infected by virus suspended in milk and heated at the same temperature. Ice cream protected the virus to such an extent that 100 per cent of the animals inoculated with virus heated at 61.7° C. became ill, and even 21 per cent came down at 65.2° C. The protective effect of cream was manifested to an even greater degree in the case of Tex.–1 virus: 100 per cent of the mice given virus heated at 65.2° C. were infected, 75 per cent at 67.7° C., and even 16.7 per cent at 71.1° C.

2. High-temperature short-time method: As the results in Table 3 show, heating at 71.1° C. for 15 seconds was adequate for the inactivation of the Conn.–5 strain suspended in water and in milk; in fact, inactivation was attained even when the holding time was only 11 seconds. The Tex.–1 strain suspended in water was also inactivated under these conditions. However, in the presence of milk the Tex.–1 virus resisted thermal destruction even when held at 71.1° C. for 20 seconds; however, virus was inactivated by increasing the holding period to 30 seconds.

The Conn.–5 strain in cream remained infectious for mice after it had been heated at 71.1° C. for 20 seconds; an additional 10 seconds were necessary to inactivate the virus. The Tex.–1 strain in the presence of cream resisted thermal inactivation to a much greater extent; virus was readily detected when heated at 71.1° C. up to 60 seconds, and a trace of virus persisted even after 75 seconds.

Ice cream apparently was not as effective as cream in protecting virus against thermal inactivation. After being heated at 71.1° C. for 15 seconds, Conn.–5 virus was barely detectable, and none was found after a holding period of 20 seconds; 71.1° C. for 15

seconds was sufficient to destroy the Tex.–1 strain.

Effect of Dilution on the Thermostability of Tex.–1 Virus—1. Holding method: It will be recalled that higher temperatures were necessary to inactivate Tex.–1 virus suspended in cream and ice cream than were necessary for Conn.–5 virus. The question arose whether this difference might be due to inherent properties of the two viruses or merely to the difference in the concentration of virus present in the heated samples. To determine what effect dilution would have on the apparent greater thermostability of Tex.–1 virus, suspensions, prepared as described under "Materials and Methods," were diluted 100-fold with the medium in which the virus was already suspended and then heated.

That dilution of Tex.–1 virus reduces its thermostability is readily evident from the results in Table 4. When Tex.–1 virus was diluted sufficiently so that it was equal in concentration to that of the Conn.–5 virus, it had the same heat susceptibilities. In contrast to the more concentrated suspensions, Tex.–1 virus diluted either in water or in cream was inactivated at the "standard" temperature for milk pasteurization, namely, 61.7° C. for 30 minutes. Ice cream still influenced the thermal inactivation of diluted Tex.–1 virus to the extent that almost all (94 per cent) of the mice inoculated with virus heated at 61.7° C. came down with disease. An increase of the temperature to 65° C., however, sufficed to inactivate the virus so that none was detectable.

2. High-temperature short-time method: As shown in Table 4, Tex.–1 virus diluted 100-fold in milk or cream was inactivated at a lower temperature

Table 4—Effect of Virus Dilution on the Thermostability of Texas-1 Virus

Dilution of Virus	Temperature °C.	Temperature °F.	Time (Minutes)	Phosphatase Test* Milk	Phosphatase Test* Cream	Phosphatase Test* Ice Cream	Fate of Test Mice: Water	Fate of Test Mice: Cream	Fate of Test Mice: Ice Cream
10^{-1}	71.1	160	30	..	0.6	0.7	..	1/6(1)	0/16
	67.7	154	30	..	0	0	..	9/12(8)	0/9
	65	149	30	..	0	0	..	9/9(6)	2/9(1)
	61.7	143	30	..	1.8	0	0/8	16/16(8)	16/16(12)
10^{-3}	71.1	160	30	..	0.6	0.7	..	0/23	0/25
	67.7	154	30	..	0	0	..	0/20	0/22
	65	149	30	..	0	0	0/15	0/23	0/20
	61.7	143	30	..	1.8	0	0/15	0/20	18/19(10)
			(Seconds)				Water	Milk	Cream
10^{-1}	71.1	160	15	..	..	..	0/38	5/33(4)	15/35(17)
10^{-3}	79.5	175	15	..	0	..	..	..	0/14
	76.7	170	15	..	0	..	..	..	0/52
	73.9	165	15	..	0	..	..	..	0/49
	72.5	162.5	15	0.4	..	..	..	0/22	..
	71.1	160	15	14.4	0.6	..	..	0/21	0/55
	69.7	157.5	15	48.0	..	..	0/21	0/18	..
	68.3	155	15	>60.0	..	..	0/21	0/15	..

* Gamma phenol per 0.5 ml.

Table 5—Effect of Elevated Temperatures on the Thermostability of Mouse-Passaged Coxsackie Viruses

Strain	Dilution	Temperature °C.	Temperature °F.	Time (Seconds)	Phosphatase Test * Milk	Phosphatase Test * Cream	Fate of Test Mice: Water	Milk	Cream
Tex.–1	10^{-1}	79.5	175	15	..	0	..	..	3/38(1)
		76.7	170	15	..	0	..	..	8/50(6)
		73.9	165	15	0.6	0	..	0/17	6/14(2)
		72.5	162.5	15	0.4	..	..	0/22	..
		71.1	160	15	14.4	0.6	0/38	2/20(2)	10/20(7)
Conn.–5	10^{-1}	79.5	175	15	..	0	..	..	6/57(6)
		76.7	170	15	..	0	..	..	3/28(2)
		73.9	165	15	..	0	0/24	..	14/46(7)
		71.1	160	15	14.4	0.6	0/24	0/14	13/27(5)

* Gamma phenol per 0.5 ml.

(68.3° C.) than is usually considered "standard" for HTST, namely, 71.1° C.

Effect of Elevated Temperatures on the Thermostability of Coxsackie Virus —High-temperature short-time method: In the previous experiments of this type (Table 3) in which the temperature was held constant and the time varied, Tex.–1 virus suspended in milk and cream was not inactivated at "standard" conditions; virus in milk was inactivated only after being held for 30 seconds at 71.1° C. and virus in cream not even after 75 seconds. Experiments were therefore carried out in which the time was held constant (15 seconds) and the temperature varied from 71.1° C. to 79.5° C. Again it was found that virus was protected by milk and to a greater extent by cream, so that heating at 71.1° C. did not suffice to inactivate all the virus present in a 10^{-1} concentration of infected tissues (see Table 5). A temperature of 72.5° C. provided enough heat to destroy Tex.–1 virus suspended in milk. However, even a temperature of 79.5° C. was not enough to inactivate completely Tex.–1 virus suspended in cream. The same pattern of resistance to thermal inactivation was obtained with Conn.–5 virus suspended in cream.

Recapitulation

Human Coxsackie viruses as present in fecal matter, whether suspended in water, milk, or cream, were destroyed at milk pasteurization temperature, 61.7° C. (143° F.) for 30 minutes. Virus suspended in ice cream mix, however, had to be heated at 65° C. (149° F.) for 15 minutes before inactivation was achieved. Thermal inactivation in all media occurred also when the viruses from human sources were heated at a temperature of 71.1° C. (160° F.) for 7.5 seconds.

Coxsackie viruses in mouse-passaged lines (Conn.–5 and Tex.–1), when suspended in water, survive 50° C. for 30 minutes. However, they are inactivated at 55° C. when heated for 10 minutes.

Mouse-passaged Coxsackie viruses suspended in water or milk were destroyed at the pasteurization temperature of 61.7° C. for 30 minutes. In cream, Conn.–5 virus had to be heated to 65.2° C. (149.3° F.) for 30 minutes and in ice cream to 62.7° C. (145° F.) for

30 minutes before it was inactivated. Tex.–1 virus in cream was first inactivated at 72.1° C. (161.8° F.) for 30 minutes and in ice cream at 67.7° C. (153.8° F.) for 30 minutes. The latter virus survived 71.1° C. when heated in cream and 65.2° C. when heated in ice cream for 30 minutes.

Conn.–5 virus in water or in milk was inactivated by high-temperature short-time pasteurization, 71.1° C. (160° F.) for 15 seconds. Tex.–1 virus in water was also destroyed by this method of pasteurization; however, in milk the virus survived for 20 seconds, but was inactivated after heating for 30 seconds.

In the presence of cream, Conn.–5 virus survived 71.1° C. (160° F.) for 20 seconds, but was inactivated after a holding period of 30 seconds; in the presence of ice cream it was destroyed after 20 seconds. In cream, Tex.–1 virus was viable after heating for 60 seconds and a trace was still detectable when held for as long as 75 seconds; in ice cream, however, virus was destroyed at the "standard" holding time of 15 seconds.

Milk, cream, and ice cream afford the Coxsackie viruses some protection during the 30-minute holding method of pasteurization. Milk prevented destruction of human and mouse-passaged virus at 55° C. (131° F.). Cream protected Conn.–5 at 62.7° C. (145° F.) and Tex.–1 virus at 71.1° C. (160° F.). Ice cream allowed human Coxsackie virus to survive at 60° C. (140° F.), Conn.–5 at 61.7° C. (143° F.), and Tex.–1 virus at 65.2° C. (149.3° F.).

The dairy products also protected the viruses treated by the high-temperature short-time method. Tex.–1 virus in milk survived heating at 71.1° C. (160° F.) for 20 seconds. Conn.–5 virus in cream was infectious after 20 seconds and Tex.–1 virus after 75 seconds. Conn.–5 virus in ice cream was detectable after 15 seconds.

Considering 61.7° C. for 30 minutes as the "standard" pasteurization of milk, there was a margin of safety of less than 7° C. (or 12° F.) for human and mouse-passaged viruses. With 71.1° C. for 15 seconds as the "standard" for the high-temperature short-time pasteurization of milk, there was a safety factor of at least 7.5 seconds for human Coxsackie viruses and at least four seconds for Conn.–5 virus, but none for Tex.–1 virus.

Dilution of Tex.–1 virus 100-fold reduced its capacity to withstand heating so that when suspended in water and in cream it was inactivated by heating for 30 minutes at 61.7° C. Ice cream afforded more protection to the diluted virus, in that it survived heating at 61.7° C., but not at 65° C., for 30 minutes. The diluted virus in water, milk, or cream was destroyed at 71.1° C. for 15 seconds.

With the time constant (15 seconds), an increase of temperature to 72.5 ° C. sufficed to inactivate a 10^{-1} concentration of Tex.–1 virus in milk. Cream protected both Tex.–1 and Conn.–5 viruses to such an extent that neither agent was completely destroyed after being heated at a temperature as high as 79.5° C.

Discussion

It is apparent that under the conditions of our experiments the holding method of pasteurization is adequate for the inactivation of certain Coxsackie viruses suspended in milk, whether their source be human stools or mouse-passaged lines. It should be emphasized that these experiments were performed with large amounts of virus, most likely in far greater excess than one would expect to find in nature. It is also evident that the high-temperature short-time method for the pasteurization of milk is adequate for the thermal inactivation of at least the human and

Conn.–5 (Type B1) viruses. This method of HTST pasteurization as devised in our laboratory, however, is not capable of inactivating the Tex.–1 strain (Type A4) suspended in milk; in this instance the holding time at 71.1° C. had to be increased to 30 seconds, twice the "standard" holding period, before inactivation occurred. It should be pointed out that since this work was done the recommended minimum temperature for HTST pasteurization has been increased to 71.7° C.

If pasteurization by heating at 61.7° C. for 30 minutes or 71.1° C. for 15 seconds, which is generally applied to milk, is used for cream and ice cream, then it is obvious that neither condition suffices for the inactivation of mouse-passaged virus suspended in the latter media. By the holding method virus suspended in cream survived heating for 30 minutes at the elevated temperature of 71.1° C. and in ice cream to 65.2° C. Thus, if one were to accept as a standard the minimum temperature of 62.7° C., which is used by some dairies for the pasteurization of cream, it is obvious that this temperature might fall short of inactivating virus having the thermostability of mouse-passaged virus. Conditions are somewhat different for ice cream, since for it heating at 68.3° C. for 30 minutes or at 79.5° C. for 25 seconds are the present recommended minimum conditions for pasteurization, and virus is inactivated at these temperatures and times.

The present experiments reveal that substances like milk and its products protect virus against destruction by heat. The results obtained with these Coxsackie viruses are similar to those found previously for poliomyelitis virus.[7] Similar to the experiments with poliomyelitis virus, cream proved to be the most effective protective agent of the dairy products studied; with a holding time of 30 minutes, virus heated in cream was able to withstand 17° C. more heat than virus suspended in water. Protection of virus by cream was striking by the high-temperature short-time method, a trace of virus persisting even after it was held at 71.1° C. for 75 seconds; this may be contrasted with 11 seconds sufficient to destroy virus suspended in water. With a holding time of 15 seconds, both the Tex.–1 and Conn.–5 mouse-passaged strains could be detected in cream after treatment at 79.5° C.

Conclusions

Coxsackie viruses as present in human stools underwent thermal inactivation when heated at 55° C. for 15 minutes or at 71.1° C. for 15 seconds when they were suspended in water. Mouse-passaged viruses (Conn.–5 strain of Type B1 and Tex.–1 strain of Type A4) in water suspension were also inactivated at these temperatures.

Dairy products, such as milk, cream, and ice cream mix, offer a measure of protection to these viruses against thermal inactivation. However, when suspended in milk, the minimum conditions recommended for pasteurization, 61.7° C. for 30 minutes or 71.1° C. for 15 seconds, proved adequate for the inactivation of the fecal strains of Coxsackie virus. The holding method of pasteurization provided sufficient heat for the destruction of the two mouse-passaged strains suspended in milk, but the high-temperature short-time method as used in these experiments did not.

Both methods of pasteurization were found capable of inactivating the human Coxsackie viruses suspended in cream. A temperature of 65° C. for 30 minutes was required for the inactivation of one of these viruses in ice cream. The complete inactivation of the mouse-passaged viruses in cream was not attained under the pasteurization conditions employed.

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