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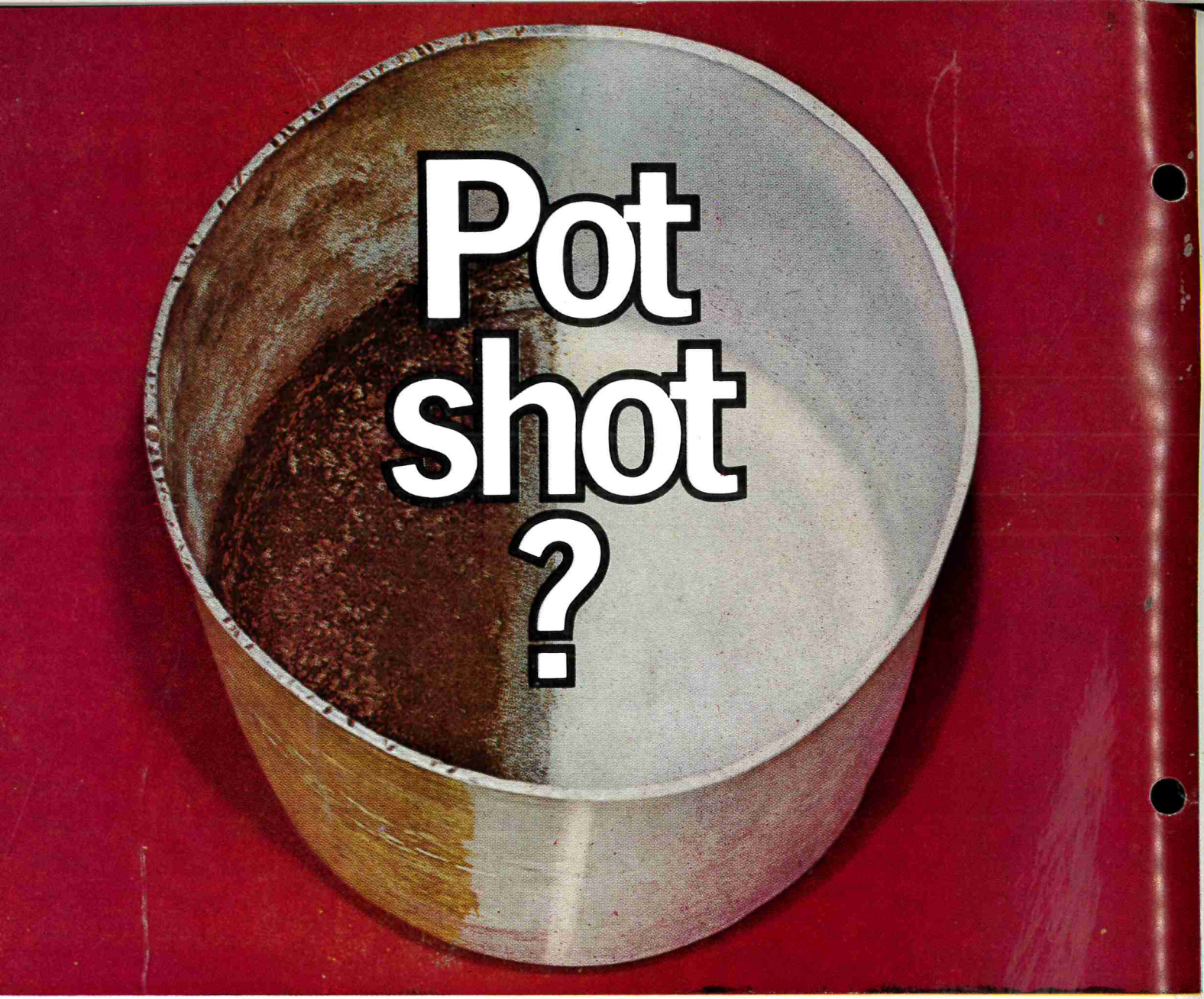

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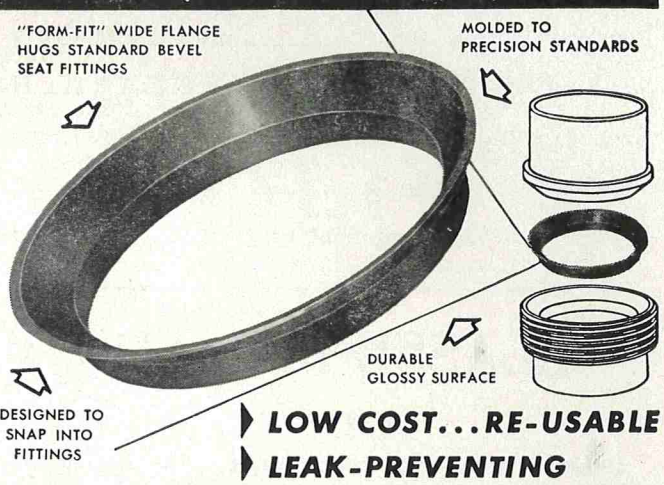

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LIPOLYTIC AND PROTEOLYTIC ACTIVITY OF ENTEROCOCCI AND LACTIC GROUP STREPTOCOCCI ISOLATED FROM YOUNG CHEDDAR CHEESE[1]

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(Received for publication March 27, 1970)

Abstract

Lipolytic and proteolytic screening techniques were applied to cultures isolated from young Cheddar cheese manufactured in 10 Iowa cheese plants. Twenty-one cultures were selected for study. These included 16 enterococci and 5 lactic group streptococci. These strains were examined for lipolytic activity when grown in skimmilk, cream, and skimmilk containing tributyrin; changes in proteolysis index, plate counts, and pH in skimmilk incubated at 7, 15, 21, and 32 C also were determined. And, combinations of enterococci and lactic streptococci were studied.

One-half of the *Streptococcus durans* strains frequently produced as much as 10 times more acetic acid than the others; the five strains of lactic streptococci consistently produced the lowest quantities of acetic acid. Compared with enterococci, except for *Streptococcus faecalis* var. *liquefaciens*, the lactic streptococci were more proteolytic, produced lower pH values, and had less viability at 15, 21, and 32 C. Enterococci other than S. *faecalis* var. *liquefaciens* were not proteolytic. All cultures showed tributyrinase activity; enterococci were the most active. Combining enterococci and lactic streptococci produced anomalous results.

After lactose degradation, lipolysis and proteolysis are presumed to be the principal changes responsible for developing Cheddar cheese flavor. Peterson et al. *(18)* have shown that lipolytic and proteolytic enzymes present during cheese ripening probably are of bacterial origin. The number and specificity of bacteria in the milk and curd should, therefore, predetermine kinds and degree of lipolysis and proteolysis subsequently occurring.

Clark and Reinbold *(4)* showed enterococci may constitute about one-half of the low-temperature flora of young Cheddar cheese; 56% of the enterococci they found were *Streptococcus durans*. Only 13.0% of the low-temperature flora were lactic group streptococci.

The ubiquity, wide growth-temperature range, and high tolerance of heat, salt, and acid of enterococci could render them important in Cheddar cheese flavor formation. Indeed, *Streptococcus faecalis* was used as a supplemental starter in Cheddar cheese manufacture by Tittsler et al. *(20)*, although no benefit was reported. On the other hand, Dahlberg and Kosikowsky *(7)* attributed improvement in Cheddar cheese body and flavor to use of *S. faecalis*. *Streptococcus durans* has been used in short-time Cheddar cheese making *(5, 22)*, although Czulak and Hammond *(6)* later recommended use of *Streptococcus thermophilus* for faster acid production. These investigations and other unreported commercial trials were made with single strain *S. faecalis* or *S. durans* selected largely on ability to produce acidity during cheese making.

Breed et al. *(3)* have not included lipolytic properties of the streptococci in their descriptions. Long and Hammer *(16)*, however, noticed strain specificity in a study of *Streptococcus liquefaciens*. Milk fat and cottonseed oil were not hydrolyzed, but 64 cultures attacked tripropionin and tributyrin. Wolf *(23)* and Oterholm et al. *(17)* also have demonstrated lipolysis by streptococci.

Other workers *(2, 9, 10, 16, 19)* have studied the proteolytic activity of enterococci, lactic streptococci, or both.

Our investigation was designed to study the proteolytic and lipolytic characteristics of enterococcus and lactic strains of streptococci in pure and mixed cultures. It was believed that cultures could be selected for Cheddar cheese manufacture on the basis of these characteristics.

Methods and Materials

General procedure

Cultures. One hundred and seven cultures of enterococcus and lactic group streptococci isolated from 41 young Cheddar cheeses from 10 Iowa cheese plants *(4)* were used in this study.

Single strain studies. Sixty-two enterococcus and lactic group streptococci were screened for lipolytic activity on 6 different substrata. Nile blue sulfate agar plates *(14)* were incubated at 21 C; readings were made at 8 and 32 days.

Simultaneously, 107 cultures were tested for proteolysis in skimmilk using an Orange G dye-binding technique *(13)*. Skimmilk cultures were incubated at 32 C; duplicate readings were made after 2, 6, 14, and 30 days.

On the basis of the first screening, 21 cultures were selected to adequately represent different taxonomic groups for study. The subsequent intensive study included determina-

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[1]Journal Paper No. J-6544 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1188.
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tion of volatile fatty acids (VFA) produced by each of the 21 cultures in skimmilk, cream, and skimmilk containing 4% tributyrin. Incubation was at 21 C; VFA measurements were made at 4, 16, and 32 days. Plate counts (PC) and pH determinations were made on all cultures at 4 and 16 days, and repeated at 32 days only on the skimmilk-tributyrin medium.

In addition, proteolysis indices (PI), PC, and pH changes of skimmilk cultures incubated at 7, 15, 21, and 32 C after 4, 16, and 32 days of incubation were determined.

Mixed strain studies. After the single strain studies, 7 culture combinations were tested for VFA, PC, and pH in skimmilk, cream, and skimmilk plus tributyrin incubated at 21 C for 4, 16, and 32 days.

Temperature effect studies. To simulate temperature changes during cheese making and to determine the possible effect of temperature changes, skimmilk cultures of the same 7 combinations were incubated, consecutively, at 32 C for 1 day, 21 C for 1 day, and at 7 C for 30 days. Proteolysis indices, PC, and pH were determined at the end of 4 days and the total 32-day incubation period.

Lipolytic activity tests

(a) Screening technique on Nile blue sulfate agar. Tripropionin, tributyrin, tricaproin, tricaprylin, triolein, and milk fat were stained with Nile blue sulfate according to the method of Knaysi *(14)*. One ml each of the sterile, stained substrate was mixed with 100 ml of special Trypticase-soy agar *(4)*. Immediately before each mixture attained gelation, plates were poured to obtain better fat dispersion. After streaking with culture, the plates were sealed with 1.75-inch wide rubber bands to prevent contamination and moisture loss and were incubated at 21 C. Changes in color, shape, and disappearance of fat globules were recorded.

(b) Determination of volatile fatty acids. Portions of sterile, reconstituted skimmilk containing 11% nonfat milk solids and 1% Tween 80, cream containing 12% milk fat, and reconstituted skimmilk containing 1% Tween 80 and 4% tributyrin were inoculated with fresh litmus milk transfers of the cultures. Five grams of each well-mixed, incubated culture were weighed into a test tube, and 0.05 ml of 10% aqueous methyl cellosolve was added as an internal standard. The culture was made acidic with 0.25 ml concentrated HCl, mixed, and centrifuged. The aqueous layer was used to measure acetic, propionic, and butyric acids by gas chromatography with a hydrogen flame detector as described by Hammond and Reinbold *(12)*. Peak areas were measured with the disc chart integrator and corrected for background. In each step, an uninoculated control with each of the 3 media was included. Amounts of the VFA were calculated by the formula:

$$C_x = \frac{M_s A_x}{M_x A_s} C_s$$

where M is the area of methyl cellosolve peak, A is the area of the acid peak, x refers to the unknown, C is concentration in mg/100 g, and s refers to the standard; and:

$$C^1_x = C_x - C_c$$

where C^1 is the corrected concentration and c refers to the uninoculated controls.

When examining the entire chromatogram, ghost peaks that appeared in a chromatogram following a bigger peak were discarded. In each trial, the skimmilk control was first chromatographed, followed by the cream, and, finally, the skimmilk plus tributyrin. After the introduction of the standards, or following a chromatogram with large peaks, water was injected to reduce the remaining amounts of VFA in the column.

Proteolytic activity test

Orange G dye solution was prepared and used according to the procedure of Hammond, Seals, and Reinbold *(13)*, except that 6.3020 g of oxalic acid dihydrate per liter was used. A single batch of 25 liters was prepared and used exclusively throughout the experiment.

Prescription bottles containing 100 ml of skimmilk autoclaved at 121 C for 13 min were inoculated with 0.5 ml of a fresh litmus milk transfer. From this, 1-ml aliquots were aseptically pipetted into sterile 20 x 125 mm screw-cap tubes. Uninoculated controls were made for each batch of skimmilk. Enough tubes were prepared to obtain quadruplicate readings at 4 days of incubation and triplicate readings thereafter. Additional culture was made for PC and pH measurements. Tests were made to determine if contamination had occurred during inoculation and transfer of the skimmilk to the tubes. If contaminants appeared on special Trypticase-soy agar plates, the corresponding series of tubes were discarded.

Plate counts and pH measurement

Procedures listed in the 11th edition of *Standard Methods for the Examination of Dairy Products (1)* were followed. Duplicate plates were poured with special Trypticase-soy agar and were incubated at 21 C for 5 days. When PCs were made along with VFA determinations, test aliquots were withdrawn directly from the flasks of incubated milk. When PCs were made to correspond with PIs, 9 ml of sterile, buffered distilled water were added to 1-ml aliquots described in the earlier sections; successive dilutions were made as required from these aliquots.

All pH measurements were made with a Beckman Zeromatic pH meter. Readings to correspond with VFA determinations were made (as with PCs) by aseptic withdrawal of test material from the bulk culture. Readings to correlate with PIs were made from separate flasks of inoculated media prepared and inoculated simultaneously with the 1-ml aliquots.

RESULTS AND DISCUSSION

Lipolytic tests on Nile blue sulfate agar

Results of the screening tests on Nile blue sulfate agar are given in Table 1. Although all cultures were able to hydrolyze tripropionin, the clear zones around the enterococcus colonies were larger after prolonged incubation. Tributyrin was less susceptible to hydrolysis but, again, the enterococci were more active than the lactic streptococci. Tricaproin and tricaprylin were less frequently hydrolyzed and triolein was not attacked at all. Table 1 shows that a few cultures seemingly hydrolyzed milk fat, but the readings are subject to question. In quite a few instances, the bacterial colonies absorbed the blue dye, this could have masked changes in the underlying material. Some of the "positive" readings resulted from fat globule distortion, but in no instances were the "positive" reactions absolutely clear. The shorter chained the triglyceride, the more distinct the readings became. Further, readings were recorded only as

TABLE 1. RESULTS OF LIPOLYSIS SCREENING TEST ON NILE BLUE SULFATE AGAR PLATES INCUBATED AT 21 C

Species	Reaction observed[a]	Substrate: Tripropionin (8 days)	Tripropionin (32 days)	Tributyrin (8 days)	Tributyrin (32 days)	Tricaproin (8 days)	Tricaproin (32 days)	Tricaprylin (8 days)	Tricaprylin (32 days)	Triolein (8 days)	Triolein (32 days)	Milk fat (8 days)	Milk fat (32 days)
S. durans	−			15	1	20	16	20	22	11	11	32	28
	+	25		18	24	3	7	3	1				2
	++	7	22		6								2
	+++	1	11		2								
		33[b]	33	33	33	23	23	23	23	11	11	32	32
S. faecalis	−			4		1	1	2	2			5	5
	+	4		1	5	1							
	++	1	3				1						
	+++		2										
		5[b]	5	5	5	2	2	2	2			5	5
S. faecalis var. *liquefaciens*	−			3		4	1	3	3	1	1	5	5
	+	3		2	3		3						
	++	3	3		2								
	+++		3										
		6[b]	6	5	5	4	4	3	3	1	1	5	5
S. faecalis var. *zymogenes*	−			1	1	1		3	2	2	2	4	4
	+	4		3	3		1		1				
	++		2										
	+++		2										
		4[b]	4	4	4	1	1	3	3	2	2	4	4
Lactic group streptococci	−	2		8	2	7	5	7	7	3	3	13	12
	+	12	1	4	9	1	3					1	1
	++		10		1								1
	+++		3										
		14[b]	14	12	12	8	8	7	7	3	3	14	14

[a]Symbols: − = no lipolysis or questionable reaction.
+ = slight positive reaction, disappearance or change in shape of colonies in immediate vicinity of bacterial growth.
++ = fat globules disappeared up to 2 mm around bacterial growth; color change.
+++ = more than 2 mm of cleared zone; distinct color change.

[b]Total number of cultures studied.

positive or negative when sufficient bacterial surface growth to produce changes had occurred. Adequate growth in the presence of longer-chained triglycerides was not always obtained. Consequently, Table 1 records progressively fewer readings from tripropionin through triolein. The Knaysi modification of the Nile blue sulfate tests thus lacked sensitivity, but did indicate that enterococci are more able to hydrolyze short-chained triglycerides than are lactic streptococci. To substantiate this, the more sensitive gas chromatographic procedure was adopted.

Chromatographic measurement of lipolysis

The production of VFA by the 21 selected cultures in skimmilk, cream, and skimmilk containing 4% tributyrin was then studied. To assure dispersion of the tributyrin, 1% Tween 80 was added to the last medium. The first medium was also fortified with Tween 80 to serve as a control. Careful testing showed that Tween 80 was neither inhibitory nor stimulatory to growth or VFA production.

Chromatographic studies (Table 2) showed that most enterococci produced more acetic acid than lactic streptococci. Strain differences with species were apparent as 50% of the *S. durans* cultures frequently produced as much as ten times more acetic acid than the others. There was little or no difference in amounts of acetic acid produced in the three media, although the presence of 4% tributyrin seemed to suppress this activity to a minor degree. The cultures that normally formed more acetic acid did not necessarily produce more butyric acid. The greater amount of milk fat in the cream medium did not lead to more acetic, propionic, or butyric acid production than in the skimmilk medium. This casts

TABLE 2. VOLATILE FATTY ACIDS, PLATE COUNTS, AND pH IN VARIOUS GROWTH MEDIA AFTER INCUBATION FOR 32 DAYS AT 21 C

Species	Culture no.	Growth medium: Skimmilk + 1% Tween 80 — Acetic	Propionic	Butyric	Cream (12% milk fat) — Acetic	Propionic	Butyric	Skimmilk + 1% Tween 80 + 4% tributyrin — Acetic	Propionic	Butyric	PC[a]	pH
		(mg/100 g of culture)										
S. *durans*	1	36(41)[b]	3	——[c]	8(17)	——	——	29(36)	——	114(145)	11,000	4.55
	2	256(567)	t[d]	——	348(773)	——	——	151(427)	——	57(139)	210,000	4.60
	3	35(112)	——	——	36(36)	t (2t)	——	73(87)	——	164(220)	240,000	4.90
	4	276(583)	——	——	378(722)	——	——	277(576)	7	215(260)	370,000	4.70
	5	19(92)	——	——	——(27)[e]	——	——	13(13)	t	182(213)	340,000	4.50
	6	245(601)	——	——	245(601)	——	——	227(407)	4	207(344)	290,000	4.62
	7	——(70)	——	——	——(71)	——	——	19(19)	——(t)	211(361)	96,000	4.58
	8	266(710)	t	——	266(710)	——	——	264(570)	——	219(277)	200,000	4.65
S. *faecalis*	9	——(131)	——	——	54(84)	——	——	77(172)	——	70(218)	180,000	4.72
	10	140(369)	——	——	107(368)	——	——	78(263)	——	82(194)	330,000	4.72
	11	89(187)	——	——	60(153)	——	——	33(216)	——	13(138)	350,000	4.70
	12	82(290)	——	——	24(75)	——	——	115(201)	——	53(116)	650,000	4.85
S. *faecalis* var. *liquefaciens*	13	50(466)	——	t	234(679)	——	t	69(307)	——	74(145)	420,000	4.75
	14	100(161)	——	t	48(155)	——	t	49(141)	——(55)	19(176)	97,000	4.50
S. *faecalis* var. *zymogenes*	15	102(551)	——	——	183(688)	——	——	63(453)	t	23(187)	240,000	5.10
	16	48(250)	——	——	64(270)	——	——	117(215)	——	18(183)	180,000	4.80
Lactic group streptococci	17	——(47)	——	——	40(116)	——	——	32(115)	——	——(123)	45	4.40
	18	——(36)	——	——	——(36)	——	——	25(56)	——	——(61)	4	4.22
	19	10(44)	——	——	14(56)	11(11)	——	60(103)	t	——(14)	<1	4.30
	20	32(73)	——	——	32(73)	——	——	24(50)	——	——(15)	18	4.12
	21	——(59)	——	——	4(51)	——	——	42(58)	——(6)	37(127)	<100	4.25

[a]Plate count/ml x 10^3.

[b]Figures within parentheses represent total amount of volatile fatty acids from determinations made after 4, 16, and 32 days incubation expressed as mg/100 g of culture.

[c]Amounts present equal to or less than in uninoculated control.

[d]Trace quantities.

[e]——(27) indicates volatile fatty acid not present at 32-day determination although measurable amounts had been present earlier.

TABLE 3. CHANGES IN PROTEOLYSIS INDEX, PLATE COUNTS[a] AND pH IN SKIMMILK INCUBATED FOR 32 DAYS AT 7, 15, 21, AND CB C

Species	Culture No.	Temperature 7C PI	7C PC	7C pH	15C PI	15C PC	15C pH	21C PI	21C PC	21C pH	32C PI	32C PC	32C pH
S. durans	1	--[b]	310,000	4.95	--	510,000	4.50	0.01	350,000	4.50	0.02	<10	4.60
	2	0.02	480,000	5.07	0.02	1,300,000	4.60	0.01	280,000	4.42	0.02	<10	4.55
	3	--	120,000	5.65	--	370,000	4.80	--	270,000	4.70	0.02	240,000	4.70
	4	0.02	450,000	5.10	0.01	100,000	4.70	0.01	400,000	4.55	0.02	300	4.55
	5	0.01	440,000	5.10	0.03	1,200,000	4.65	--	20,000	4.52	0.04	<10	4.50
	6	--	230,000	5.65	--	560,000	4.80	0.01	510,000	4.52	--	8	4.88
	7	0.01	280,000	5.25	0.02	250,000	4.62	--	450,000	4.42	--	19	4.52
	8	0.02	320,000	5.00	0.01	720,000	4.73	--	600,000	4.60	0.01	11	4.52
S. faecalis	9	0.01	180,000	5.32	0.01	570,000	4.92	--	700,000	4.65	--	49	4.70
	10	--	110,000	5.40	--	160,000	4.95	0.02	400,000	4.82	0.03	610,000	4.80
	11	--	57,000	5.85	--	260,000	4.85	--	2,900,000	4.82	--	1	4.75
	12	0.01	200,000	5.47	--	610,000	5.22	--	61,000	4.75	--	900,000	4.85
S. faecalis var. *liquefaciens*	13	0.52	600,000	5.00	0.81	970,000	4.80	0.83	650,000	4.62	0.87	75	4.75
	14	0.27	53,000	4.45	0.52	1,500,000	4.48	0.61	530,000	4.42	0.61	30	4.42
S. faecalis var. *zymogenes*	15	0.02	230,000	6.30	--	550,000	5.62	--	500,000	4.97	0.01	5,900	4.96
	16	0.03	250,000	5.55	0.02	520,000	5.18	--	370,000	4.82	0.02	2,200	4.82
Lactic group streptococci	17	--	360,000	5.00	0.01	70,000	4.35	0.02	15,000	4.25	0.03	<1	4.45
	18	0.05	63,000	4.25	0.09	30	4.45	0.12	<1	4.15	0.14	<1	4.22
	19	--	440,000	4.98	0.01	120,000	4.32	0.03	190,000	4.28	0.03	<10	4.32
	20	--	21,000	4.85	0.08	270,000	4.28	0.12	260,000	4.10	0.11	<10	4.20
	21	0.03	280,000	4.65	0.03	290,000	4.28	0.04	<10	4.25	0.02	<10	4.45

[a]Plate count/ml x 10^3.

[b]Amount of proteolysis present equal to or less than in uninoculated control.

TABLE 4. VOLATILE FATTY ACIDS, PLATE COUNTS, AND pH PRODUCED BY MIXED CULTURES OF ENTEROCOCCI AND LACTIC GROUP STREPTOCOCCI IN VARIOUS GROWTH MEDIA AFTER INCUBATION FOR 32 DAYS AT 21 C

Culture	Growth medium: Skimmilk + 1% Tween 80 — Acetic	Propionic	Butyric	Cream (12% milk fat) — Acetic	Propionic	Butyric	Skimmilk + 1% Tween 80 + 4% tributyrin — Acetic	Propionic	Butyric	PC[a]	pH
	(mg/100 g of culture)										
S. durans 6 + Lactic gp. strep. 19	45(180)[b]	t[c](1)	--[d]	57(91)	t	--	61(175)	t(7)	19(107)	2,800	4.28
S. faecalis 9 + Lactic gp. strep. 19	59(137)	--(1)[e]	--	29(59)	--(t)	--	19(63)	--	13(44)	1,300	4.28
S. f. var. liquef. 13 + Lactic gp. strep. 19	117(328)	--(t)	--	40(133)	--(t)	--	40(198)	--(t)	15(114)	1,900	4.30
S. durans 6 + *S. faecalis* 9	35(153)	--(t)	--	71(138)	--(t)	--	84(195)	--(12)	28(28)	310,000	4.68
S. durans 6 + *S. f.* var. *liquef.* 13	174(281)	--	--	171(387)	--	--	148(320)	--	70(111)	490,000	4.80
S. durans 6 + Commercial starter	52(219)	--(4)	t	103(290)	--(t)	t	137(316)	--(1)	12(97)	4,900	4.22
S. faecalis 9 + Commercial starter	116(256)	--(t)	t	118(232)	--(t)	t	99(284)	--(1)	41(77)	650	4.25

[a]Plate count/ml x 10^3.

[b]Figures within parentheses represent total amount of volatile fatty acids from determinations made after 4, 16, and 32 days incubation expressed as mg/100 g of culture.

[c]Trace quantities.

[d]Amounts present equal to or less than in uninoculated control.

[e]--(1) indicates volatile fatty acid not present at 32-day determination although measurable amounts had been present earlier.

doubt upon the true lipolytic activity of the enterococci. The acetic acid formed may result from normal fermentative processes although supporting data were not obtained. The presence of tributyrin, however, greatly enhanced the liberation of butyric acid, thereby reemphasizing the greater tributyrinase activity of the enterococci and, especially, of S. *durans*. According to Forss and Patton *(11)*, acetic, butyric, and caproic acids appear to be indispensable for the flavor and aroma of Cheddar cheese. Possibly, the optimum ratio is 8:1:0.3, or the amounts 900, 110, and 35 ppm. A bacterial culture that could produce these compounds in approximately this ratio would be of value in cheese ripening provided it was not strongly proteolytic or produced undesirable flavors from other metabolic activities and large quantities of carbon dioxide. The amounts of acetic acid shown here were found in a fluid medium with properties radically different from cheese curd. Nevertheless, continuing growth of enterococci could conceivably perform this function although Dahlberg and Kosikowsky *(7)* indicated that their cheese made with S. *faecalis* contained less VFA than the negative controls. Their culture had been selected for its ability to produce lactic acid and not VFAs.

Table 2 also shows that enterococci remain viable under unfavorable culture conditions much longer than do lactic streptococci. This ability of enterococci which may be of value in Cheddar cheese ripening, has already been noted by Kosikowsky and Dahlberg *(15)*. The difference in 32-day pH values (enterococci, pH 4.5-5.10 and lactic streptococci, pH 4.12-4.40) undoubtedly is of some importance in this respect. Only the PC and pH readings at 32 days in skimmilk plus tributyrin are given in Table 2, since earlier tests showed the same trends.

Within the 4 temperatures and 3 times studied (only 32-day values are recorded in Table 3, however), there were general differences in growth and viability between the two groups of streptococci. Although not evident in Table 3, the enterococci grew only slightly better at 7 C than did the lactic streptococci. There was little difference in numbers between the groups after 4 days at 15 C, but the lactic streptococci then declined more rapidly during prolonged incubation. At 21 and 32 C, the lactic streptococci had reached maximum numbers and had declined rapidly before the first count was made at 4 days. Under the test conditions it was not evident whether the enterococci were continuing to grow or whether they had reached a near maximum level and persisted at that level under the unfavorable growth conditions. This differentiation could be important because continuing metabolic activity could be of value in cheese ripening if it would contribute to an increase of flavor compounds or even deter the growth of less desirable or deleterious microorganisms.

Streptococcus durans and S. *faecalis* showed barely measurable increases in PI, with essentially no changes at 7, 15, and 21 C and only an exceedingly minor increase at 32 C. *Streptococcus faecalis* var. *zymogenes* (only 2 cultures tested) showed no differences in proteolytic activity at the different incubation temperatures; PI changes for S. *faecalis* var. *liquefaciens* were related directly to temperature, with growth at 32 C producing earlier and greater proteolysis. Temperature increase affected lactic streptococcus PIs; higher temperatures produced higher PIs. Strain differences were evident; those cultures that produced lower pH readings were more proteolytic, except with the enterococcus cultures. In general, the enterococci neither produced as low pH values at any temperature nor did the pH drop occur as quickly.

Mixing enterococci and lactic group streptococci produced the results given in Table 4. When three different enterococci were separately combined with a low acetic-acid-producing and weak tributyrinolytic lactic streptococcus, acetic acid and tributyrinase production by the enterococci were invariably inhibited. Final pH values were representative of lactic group streptococci, and the TCs were greatly reduced. Mixing two enterococcus cultures did not reduce either viability or final pH, but did reduce anticipated acetic and butyric acid production. The use of a commercial Cheddar cheese starter also reduced PC, pH, and VFA content of the 32-day-old cultures. Trace quantities of propionic and butyric acid increased in the cream and skimmilk media. Cheesy flavors were never noticed in this or any other of our experiments.

The same mixed cultures of enterococci and lactic group streptococci were used to study effect upon PI (Table 5). To simulate cheese ripening conditions, cultures were grown at 32 C for the first day, transferred to 21 C for the second day, and were then held at 7 C until tested at 4 and 32 days after inoculation. Single-strain enterococcus and lactic group streptococcus combinations had little effect upon PI except where S. *faecalis* var. *liquefaciens* was included. In all seven trials, greatest proteolysis occurred within the first 4 days; indeed, during the remaining 28 days at 7 C, 5 cultures showed a negligible decrease in PI, meaning that activity had been only at the lower limit of testing sensitivity. The S. *faecalis* var. *liquefaciens* culture, greatly proteolytic after 4 days, continued to raise the PIs by 0.10 and 0.08 unit up to 32-day readings of 0.80 and 0.81. Changes of these magnitudes would, of course, be

TABLE 5. DIFFERENCES IN PROTEOLYSIS INDEX, PLATE COUNTS[a], AND pH PRODUCED BY MIXED CULTURES OF ENTEROCOCCI AND LACTI GROUP STREPTOCOCCI IN SKIMMILK INCUBATED FOR 1 DAY AT 32 C, 1 DAY AT 21 C, AND FOR 2 AND 30 DAYS AT 7 C

Culture	PI		PC		pH	
	days		days		days	
	4	32	4	32	4	32
S. *durans* 6 + Lactic gp. strep. 19	0.03	−−[b]	2,200	140	4.30	4.28
S. faecalis 9 + Lactic gp. strep. 19	0.02	−−	1,500	730	4.25	4.25
S.*f.* var. *liquef.* 13 + Lactic gp. strep. 19	0.70	0.80	2,900	2,600	4.65	4.70
S. *durans* 6 + S. faecalis 9	0.03	0.01	1,400	1,500	4.52	4.50
S. *durans* 6 + S.*f.* var. *liquef.* 13	0.73	0.81	2,400	1,800	4.67	4.75
S. *durans* 6 + Commercial starter	0.06	0.05	1,200	21	4.50	4.55
S. faecalis 9 + Commercial starter	0.05	0.05	290	40	4.42	4.60

[a]Plate count/ml x 10^{-6}.
[b]Amount of proteolysis equal to or less than in uninoculated control.

undesirable in Cheddar cheese ripening. Deane *(8)* has reported that the addition of 0.5% S. *faecalis* var. *liquefaciens* to a commercial starter caused cheese to develop an intense, bitter flavor and a soft, pasty body. The temperature sequence used produced notably high PCs after 4 days, and the subsequent decline in viability at 7 C was less than would have been anticipated at the unfavorable pH levels. There was little change in pH from 4 to 32 days. Three of the culture combinations stayed essentially the same, 2 increased by only 0.05 unit, and the remaining cultures increased 0.08 and 0.18 unit.

By studying a selected group of enterococcus and lactic group streptococci in pure and combined culture, we have been able to note differences between proteolytic and lipolytic characteristics of strains of the same species. Some of these strains will be used to manufacture Cheddar cheese which will be compared for differences in body, flavor, and texture.

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AFLATOXINS: PRODUCTION ON BEANS AS AFFECTED BY TEMPERATURE AND MOISTURE CONTENT

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Abstract

The suitability of dried navy, pinto, and kidney beans as substrates for the support of growth and aflatoxin production by *Aspergillus parasiticus* was investigated. Inoculated beans adjusted to 20, 25, or 30% moisture were incubated at 21, 28, or 35 C for 1, 3, or 5 weeks. Aflatoxins were produced on all substrates having 25 or 30% moisture when incubation was at 21, 28, or 35 C. Substrates containing 30% moisture and incubated at 28 C supported maximum aflatoxin production. There was a decrease in aflatoxin concentrations after these maxima were reached.

The potency of aflatoxins as carcinogenic agents to animals has resulted in a tremendous amount of research to determine the substrates on which and the environmental conditions under which aflatoxin-producing fungi are able to grow and produce toxin. Aflatoxins accumulate when certain species of aspergilli are cultured on natural substrates such as peanuts, rice, oats, corn, and other grains. Toxins are produced on fruits and fruit juices, cheeses, and aged meats. The effects of environmental factors such as temperature, moisture content, relative humidity, and atmospheric gases on aflatoxin production in some substrates has been studied in depth. Temperature is recognized as one of the most important factors affecting fungal growth as well as aflatoxin production *(1, 2, 8, 9)*. The temperature range for production is between 20 and 35 C. The ratios of aflatoxins produced at different temperatures vary *(7)*, but the greatest total quantity of toxins produced is at 25 to 28 C, depending upon the substrate. Generally, growth substrates having less than 10% moisture or water activities of less than 0.80 are unsatisfactory for the support of growth and aflatoxin production.

Freshly harvested beans may be exposed to environmental conditions, either during transport or storage, which permit growth and production of aflatoxins by aspergilli. Beans have been exposed to high moisture conditions during transport, and mold often grows on the burlap sacks and in the outer layers of beans left exposed on shipping docks and in the holds of ships. Soybeans have been reported to support aflatoxin production poorly *(3)*, whereas little is known of the ability of other dry beans which are held under various environmental conditions to support growth with subsequent production of aflatoxin by aflatoxin-producing fungi. This study was designed to investigate the interacting effects of temperature and moisture on the rate of production and total production of aflatoxins B_1, B_2, G_1, and G_2 by *Aspergillus parasiticus* on navy, kidney, and pinto beans (all varieties of *Phaseolus vulgaris*).

Materials and Methods

Growth media

Sound whole beans were dried at 110 C for 2 days to enable the adjustment of moisture content. Beans were darkened slightly and reduced in size because of the loss of water. Assuming the dried beans to have zero moisture, enough tap water was added to 40 g of the dry product in a 500-ml Erlenmeyer flask to bring the moisture content (wet weight basis) to 18, 24, or 29%. The flasks were stoppered tightly with non-absorbent cotton plugs wrapped with cheesecloth and parafilm. The bean-water mixture was allowed to stand for several hours to facilitate the absorption of water into the beans and then autoclaved for 15 min at 121 C. The sterile substrate was shaken to free the beans from each other and cooled to room temperature.

Culture preparation

Aspergillus parasiticus NRRL 2999 was cultured on potato dextrose agar slants for 7 days at 28 C before suspending the green conidia in 4.0 ml of sterile 0.005% Triton X-100 by scraping with a loop. Suspensions from several tubes were pooled, conidia were counted, and the suspensions were adjusted to 1 x 10^8 conidia per milliliter by diluting with sterile water.

Culturing A. parasiticus on bean substrates

The bean substrates (navy, kidney, and pinto) were inoculated with 0.5 ml of the conidia suspension and shaken by hand to assure an even distribution of conidia. Six flasks of each of the three bean substrates adjusted to a final moisture content of 20, 25, or 30% were incubated at each of three incubation temperatures, 21, 28, and 35 C. Duplicate substrates of each moisture content were harvested at each incubation temperature after 1, 3, and 5 weeks. The flask contents were shaken by hand once each day during the first week of incubation and every other day thereafter.

Sample extraction

The procedure for aflatoxin extraction and purification was modified from the method of Pons et al. *(6)*, to facilitate maximum extraction of the toxin from the bean substrates. The following description is for one 40-g sample. At the termination of the incubation period, the sample was steamed briefly to kill the mycelia and cooled to room temperature. Thirty milliliters of extraction solvent (acetone:water:glacial

TABLE 1. QUANTITATIVE DETERMINATION OF AFLATOXINS B_1, B_2, G_1 G_2 PRODUCED BY *Aspergillus parasiticus* NRRL 2999 ON NAVY, PINTO, AND KIDNEY BEANS. BEANS WERE ADJUSTED TO 20, 25, OR 30% MOISTURE AND INCUBATED AT 21, 28, OR 35 C FOR 1, 3, OR 5 WEEKS AFTER INOCULATION. ALL VALUES LISTED HAVE BEEN CORRECTED FOR PERCENT RECOVERY AND AFLATOXIN PRESENT IN THE ORIGINAL INOCULUM. A LINE (—) REPRESENTS NO AFLATOXIN WAS DETECTED; (TR) SIGNIFIES LESS THAN 0.1 μg/g WAS DETECTED.

Navy beans							Pinto beans							Kidney beans						
Incubation time (weeks)	Moisture (%)	Temperature (C)	Aflatoxin (μg/g)				Incubation time (weeks)	Moisture (%)	Temperature (C)	Aflatoxin (μg/g)				Incubation time (weeks)	Moisture (%)	Temperature (C)	Aflatoxin (μg/g)			
			B_1	B_2	G_1	G_2				B_1	B_2	G_1	G_2				B_1	B_2	G_1	G_2
1	20	21	—	—	—	—	1	20	21	—	—	—	—	1	20	21	—	—	—	—
1	20	28	—	—	—	—	1	20	28	—	—	—	—	1	20	28	—	—	—	—
1	20	35	—	—	—	—	1	20	35	—	—	—	—	1	20	35	—	—	—	—
3	20	21	—	—	—	—	3	20	21	—	—	—	—	3	20	21	—	—	—	—
3	20	28	TR	—	TR	—	3	20	28	TR	—	TR	—	3	20	28	TR	—	TR	—
3	20	35	—	—	—	—	3	20	35	—	—	—	—	3	20	35	—	—	—	—
5	20	21	—	—	—	—	5	20	21	—	—	—	—	5	20	21	—	—	—	—
5	20	28	TR	—	TR	—	5	20	28	TR	—	TR	—	5	20	28	TR	—	TR	—
5	20	35	—	—	—	—	5	20	35	—	—	—	—	5	20	35	TR	—	TR	—
1	25	21	TR	—	TR	—	1	25	21	0.2	TR	0.2	0.1	1	25	21	TR	—	0.1	—
1	25	28	0.7	TR	0.6	0.1	1	25	28	16.2	3.7	30.5	6.7	1	25	28	16.3	1.0	14.2	0.7
1	25	35	0.7	0.1	0.5	0.2	1	25	35	0.2	TR	TR	0.1	1	25	35	12.2	1.5	15.9	2.1
3	25	21	0.8	TR	0.9	TR	3	25	21	0.4	0.2	0.5	0.2	3	25	21	0.1	TR	0.2	0.1
3	25	28	1.6	0.2	1.5	0.3	3	25	28	40.9	7.3	50.0	9.8	3	25	28	20.4	6.1	94.9	5.9
3	25	35	1.2	0.3	1.4	0.4	3	25	35	2.1	0.4	1.0	0.9	3	25	35	18.3	4.9	22.3	4.5
5	25	21	0.3	0.1	0.4	0.1	5	25	21	0.2	0.1	0.2	0.1	5	25	21	0.1	TR	0.1	0.1
5	25	28	0.4	0.2	0.7	0.3	5	25	28	32.6	6.1	45.2	7.3	5	25	28	5.6	3.1	30.5	4.9
5	25	35	1.0	0.2	1.1	0.5	5	25	35	1.8	0.2	0.7	0.6	5	25	35	5.0	2.7	6.3	3.7
1	30	21	1.6	TR	0.8	TR	1	30	21	0.1	TR	0.2	TR	1	30	21	0.1	—	0.2	—
1	30	28	158.5	18.3	162.7	36.6	1	30	28	0.3	0.1	0.2	0.1	1	30	28	50.1	24.4	44.6	15.9
1	30	35	67.9	11.0	61.0	12.2	1	30	35	TR	TR	0.1	TR	1	30	35	40.6	12.2	36.6	8.5
3	30	21	20.4	2.4	32.6	2.4	3	30	21	0.3	TR	0.4	TR	3	30	21	0.1	TR	0.2	0.1
3	30	28	122.0	12.2	135.4	20.4	3	30	28	203.3	24.4	213.4	26.8	3	30	28	202.4	73.2	304.9	36.6
3	30	35	67.8	11.0	48.8	11.0	3	30	35	14.6	9.9	125.0	9.2	3	30	35	184.2	36.5	142.3	20.4
5	30	21	2.4	0.7	8.1	1.1	5	30	21	0.2	TR	0.2	TR	5	30	21	8.2	2.4	14.6	2.6
5	30	28	101.6	6.1	12.4	18.3	5	30	28	133.8	17.1	111.0	9.8	5	30	28	159.2	61.0	264.3	24.4
5	30	35	58.5	9.3	46.3	9.8	5	30	35	48.8	2.5	45.2	4.2	5	30	35	134.2	24.4	67.1	16.3

acetic acid, 425:75:4, v/v/v) were added to the sample and, after 30 min, the mixture was blended 1 min at low speed in a Waring blendor (glass, fitted with a special Teflon seal). The sample was tranferred quantitatively back to the 500-ml Erlenmeyer culturing flask by rinsing the blender with an additional 170 ml of extraction solvent. The flasks were stoppered, held quiescently for 1 hr, and then shaken vigorously for 20 min on a Burrell Wrist Action mechanical shaker (Fisher Scientific, Pittsburgh, Pa.). The bean extract was then filtered through a folded 18.5 cm circle of Cenco No. 13250 filter paper.

Lead acetate treatment

One-hundred milliliters of the crude extract were measured into a 250-ml beaker along with 20 ml of 20% neutral lead acetate containing 0.3% glacial acetic acid, 20 ml of distilled water, and several clean boiling chips. The mixture was boiled on a steam bath until the volume was reduced to 100 ml, cooled to room temperature, and transferred quantitatively with distilled water to a 250-ml graduated cylinder. After bringing the final volume to 200 ml with distilled water, 5 g of Celite analytical filter aid (Johns Manville Corp., Manville, N.J.) were added to the mixture, stirred, and the mixtures was filtered.

Partitioning and cleanup

A 150-ml aliquot of the above filtrate was transferred to a 250-ml separatory funnel (Teflon fitted) and partitioned twice with 50 ml of chloroform. Partitioning was obtained by shaking the funnel contents vigorously for 1 min. The chloroform was drawn off through a 2-inch column of anhydrous sodium sulfate which was washed with 25 ml of chloroform after the second partitioning. The chloroform extracts were evaporated to near dryness on a steam bath and retained for the column cleanup procedure.

Approximately 2 cm of sodium sulfate were placed at the bottom of a Fischer and Porter chromatographic tube (400 mm long by 20 mm id, with coarse porosity fritted disc, Teflon fitted) and wetted with wash solvent (anhydrous diethyl ether:hexane, 3:1, v/v). A slurry prepared by mixing 15 g of SilicAR Mallinckrodt CC-7, 100-200 mesh) with 40 ml of wash solvent was poured into the column. Another 2-cm layer of sodium sulfate was placed on top of the column. The sample extract was transferred with a Pasteur pipette to the column by dissolving it in approximately 5 ml of chloroform. The column was washed with 150 ml of wash solvent and the eluate was discarded. Finally, the aflatoxins were removed from the column by eluting with 200 ml of elution solvent (chloroform:acetone, 4:1, v/v). The eluate was evaporated to near dryness on a steam bath, and the toxins were dissolved in enough chloroform to permit quantitative transfer to a vial. After evaporation to dryness, the vials were flushed with nitrogen and stored at −20 C.

Quantitation

The dry extract was dissolved in 0.5 ml of chloroform and 2-, 6-, and 10-μl aliquots were spotted, along with 1-, 3-, and 5-μl aliquots of an aflatoxin standard containing 1.0 μg B_1, 0.3 μg B_2, 1.0 μg G_1, and 0.3 μg G_2 per ml, on thin layer chromatography plates (Adsorbosil-5, 20 x 20 cm, 250μm layer, Applied Science Laboratories, State College, Pa.). The plates were developed in chloroform: acetone:2-propanol (34:5:1, v/v/v) in an unlined, unequilibrated tank (Desaga Multiplate Glass Tank 25-10-25, Brinkman Instruments, Inc., Westbury, N. Y.). The plates were air dried in the dark for 15 min upon completion of development, and then observed under long-wave ultraviolet light to visually estimate the concentration of aflatoxins in the sample extract spot. Dilutions of the sample were made when high concentrations of aflatoxins made visual estimation difficult or impossible. Calculation of the aflatoxin concentrations was made according to the procedure of Pons et al. *(6)*.

RESULTS AND DISCUSSION

Controls and precautions

The per cent recovery of aflatoxin from beans was determined to be 82% using the analytical procedure described above. Less than 10 ng/g of any one aflatoxin was found to be present in the substrate upon inoculating with the conidia suspension. Although Pons *(5)*, recently pointed out that there is a high degree of correlation between visual and densitometric estimation of aflatoxins in cottonseed products, visual estimation is still regarded as the less sensitive of the two methods of quantitation. Because the methods of extraction and purification were not designed specifically for a bean substrate and because, in some instances, no aflatoxins were detected in the inoculated medium, the concentrations of aflatoxins as calculated from the method cited above and reported in Table 1 have been adjusted to the nearest 0.1 μg/g. It was our objective to demonstrate the suitability of beans containing various moisture contents and held at various temperatures as substrates for aflatoxin production. To prevent the uncontrollable bacterial degradation during incubation in the bean substrates which had been adjusted to high moisture levels, the bean substrates were sterilized by heat. Although autoclaving may have altered the substrates with regard to their ability to support growth and/or aflatoxin production, this procedure was preferred over the uncontrolled microbial growth that would occur in non-sterile substrates. The data presented in Table 1 are only suggestive of the substrate's potential to support such production. If the maximum yields of aflatoxins were desired, the extraction, purification, and quantitation procedures would have to be adapted specifically with the chemical composition of beans in mind.

There were fluorescing compounds, either natural constituents of beans or metabolic products of the fungus, present on the thin layer chromatograms. Most did not interfere with quantitative estimation of the toxins but, as a precaution to detect the presence of possible interfering substances, samples were selected at random and chromatograms were developed using chloroform:acetone (85:15, v/v), chloroform:methanol (95:5, v/v), and toluene:ethyl acetate:formate (50:40:10, v/v/v) as solvent systems. Internal standards were also run. No interfering substances were detected by this procedure.

Light sensitivity of aflatoxins demanded that the samples be kept in the dark as much as possible during incubation and that the extraction procedure be

carried out in a dimly lighted room. Standards were shielded from direct light and stored in sealed volumetric flasks under a chloroform atmosphere at 4 C when not in use. Before thin layer chromatographic analyses, standards were allowed to warm to room temperature. All glassware was rinsed with 5% NaOCl after use.

Aflatoxin yields

All data are presented in Table 1. Only 4 of the 8 known aflatoxins were quantitated. Each of the bean substrates was shown to support growth and toxin production by *A. parasiticus*. Maximum yields obtained were 162.7, 213.4, and 304.9 μg/g aflatoxin G_1 and 158.5, 203.3 and 202.4 μg/g aflatoxin B_1 on navy, pinto, and kidney beans, respectively. Generally, total production was best on kidney beans and poorest on navy beans. Storage of sample extracts from navy beans was considerably longer than kidney beans. Instability of the toxins because of their susceptibility to oxidation or to some other form of chemical alteration could have contributed to generally lower quantities detected in navy beans. Maximum yields were obtained in all instances when the substrate had 30% moisture and was incubated at 28 C. The rate of production was somewhat different for navy beans than for pinto and kidney beans. Maximum yields for the latter two substrates were determined after 3 weeks incubation while growth on navy beans resulted in maximum aflatoxin concentrations after 1 week. There was a decrease in aflatoxin concentration which could have been a result of degradation caused by the fungus or by bean components after these maxima were reached. Beans containing 20% moisture supported limited growth of the fungus as evidenced by visual examination during the incubation period. As a result of insufficient moisture, very little, and sometimes no, toxin was produced on these beans. Thus, there should be little risk of aflatoxin contamination in dry beans dried to the normal moisture levels of 10 to 12% and stored so that there will be no increase in moisture content.

No attempt was made to control or analyze the atmospheric gases present in the culturing flasks during the incubation period. Landers et al. *(4)*, reported that aflatoxin production in peanut kernels decreased with increasing concentrations of CO_2 from 0.03 to 100%. These investigators found in general, that a reduction of 0_2 concentration decreased the amounts of aflatoxin. It is speculated that the lack of a fresh air supply caused by the use of parafilm wrapped cotton stoppers in the culturing vessels used in our experiments resulted in an increase in CO_2 concentration and a decrease in 0_2 concentration, thereby inhibiting maximum potential aflatoxin yields on the bean substrates.

The evidence presented here clearly demonstrates that precautions should be taken to protect harvested beans from abnormal abuse prior to processing to assure limited fungal growth and possible aflatoxin contamination. Although beans normally contain 14 to 18% moisture at harvesting, sacks of beans may be subjected to environmental conditions during storage or transport which would elevate moisture content. Moisture condensation on surfaces of beans stored or transported in poorly ventilated containers could conceivably allow surface growth of aflatoxin-producing fungi. Such beans might develop off odors and/or colors which would make them suspect of fungal contamination and worthy of aflatoxin analysis.

ACNKOWLEDGMENTS

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THE EFFECT OF HEAT TREATMENT OF MILK UPON THE HYDROLYZABILITY OF LACTOSE BY THE ENZYME LACTASE[1]

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Abstract

Heat treated, condensed skimmilk and nonfat dry milk (NDM) were treated with lactase enzyme obtained from *Saccharomyces fragilis*. The effect of heat treatment on lactose hydrolysis was determined. Forewarming treatments of 62.8, 68.3, 73.9, 79.5, and 85.0 C resulted in 17, 17, 100, 59, and 156 g, respectively, of lactose hydrolyzed. Concentrates with forewarming treatments of 62.8, 73.9, and 85.0 C for 30 min were given additional heat treatments of 62.8, 73.9, or 85.0 C for 20 min. Activity was lower in all concentrates given the double heat treatment, except those heated at 85.0 C for 20 min after forewarming at 62.8 and 73.9 C. These showed a substantial increase in lactose hydrolysis. Portions of each concentrate, spray dried and reconstituted to 30% total solids, exhibited the same effect on lactose hydrolysis as the original concentrates.

Additional studies were conducted with whey and a 5% lactose plus milk salt solution. Results indicated that heating lactose in the presence of milk protein had a great effect on lactose hydrolysis.

Lactose, the major constituent of milk solids-not-fat, limits the per cent of milk solids-not-fat that can be used in ice cream, milk concentrates, pasteurized processed cheese spreads, and numerous other food and feed products. Lactose utilization is limited by its low solubility, lack of sweetness, and its laxative effect if consumed at high levels. Hydrolysis of lactose by lactase enzymes will overcome some of these limitations and permit greater usage of lactose and lactose-containing products.

The beneficial effects of partial hydrolysis of the lactose in ice cream was shown by Albrecht and Gracy *(1)*, and Stimpson *(5)* reported a similar effect in frozen concentrated milk. In both studies, a lactase enzyme produced by a lactose fermenting yeast was used and the results were reported as per cent lactose hydrolyzed. However, these investigators did not report the previous temperature history of the milk product or comment on the effect that temperature history has on the hydrolyzability of lactose.

The potential importance of heat treating lactose containing products was suggested by Van Dam, et al. *(8)*, in 1950. They reported that lactase enzyme obtained from *Saccharomyces fragilis* yielded a greater rate of lactose hydrolysis in pasteurized milk than in sterilized milk. They also found it useful to heat the milk to a high temperature prior to spray drying to accomplish complete hydrolysis of the lactose in the reconstituted product.

This study investigated the effects of forewarming temperatures and additional heat treatments applied to milk concentrates on lactose hydrolysis by yeast lactase.

Methods and Materials

On two separate occasions, raw skimmilk was obtained from the University of Wisconsin dairy plant. For each experiment, the skimmilk was divided into five lots and given forewarming treatments of 62.8, 68.3, 73.9, 79.5, and 85.0 C for 30 min. Each lot of skimmilk was then concentrated to 30% total solids. The three lots of skimmilk concentrate forewarmed at 62.8, 73.9, and 85.0 C for 30 min were divided into four sub-lots. Three of the sub-lots were given heat treatments of 62.8, 73.9, and 85.0 C for 20 min just prior to spray drying, whereas the fourth portion received no additional heat treatment. A two liter sample of each concentrate was stored at 5 C until assayed on the following day. The remainder of each lot was spray-dried in the University of Wisconsin tower dryer *(2)*, with steam heated air. The concentrate was atomized through a Spraying Systems 64-21 pressure nozzle with a pressure of 175.8 kg/cm^2. Inlet air temperatures were maintained at 176.7 $\pm$ 2.8 C. Outlet air temperatures (at the point of powder and air separation) were maintained at 98.9 $\pm$ 2.8 C.

Whey from pizza cheese made by direct acidification methods *(3)* and a 5% lactose in salt solution *(4)* also were divided into five lots and given forewarming treatments of 62.8, 68.3, 73.9, 79.5, and 85.0 C for 30 min. Each lot was then concentrated to contain 15.7% lactose, which is equivalent to the lactose content of condensed skimmilk containing 30% total solids. Concentrates were stored at 5 C until assayed the following day.

For determination of lactose hydrolysis, duplicate nonfat dry milk (NDM) samples were reconstituted to 30% total solids with distilled water. The skimmilk concentrates and the reconstituted NDM samples were adjusted to pH 6.6 with 10% KOH solution. Forty grams of each were placed in 60 ml screw cap sample bottles and immersed in a constant temperature bath at 50 C. After each sample had reached the temperature of the bath, 10 mg of a dried lactase yeast preparation *(9)* was mixed with the concentrate and held over a period of 4 hr. At the end of this time, the lactase was inactivated by heating for 15 min at 70-75 C. Lactose hydrolysis was estimated from the weight of monosaccharides liberated during the hydrolysis of the lactose in the concen-

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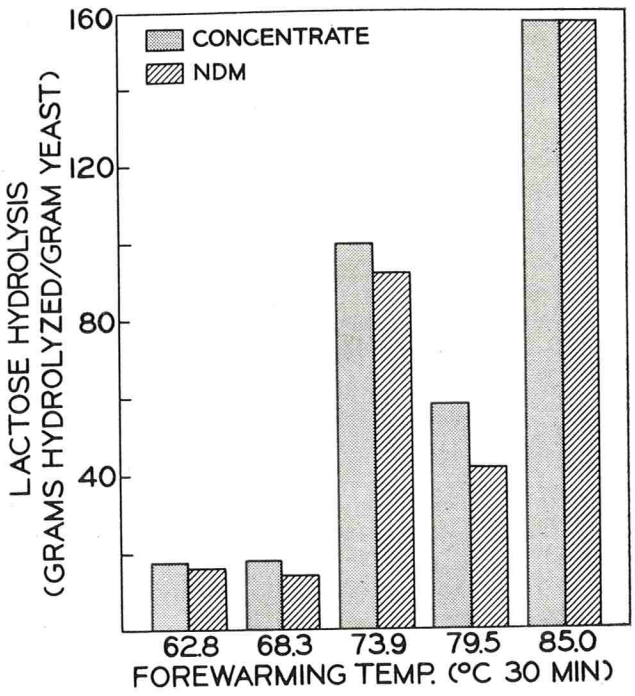

Figure 1. Effect of forewarming skimmilk on lactose hydrolysis.

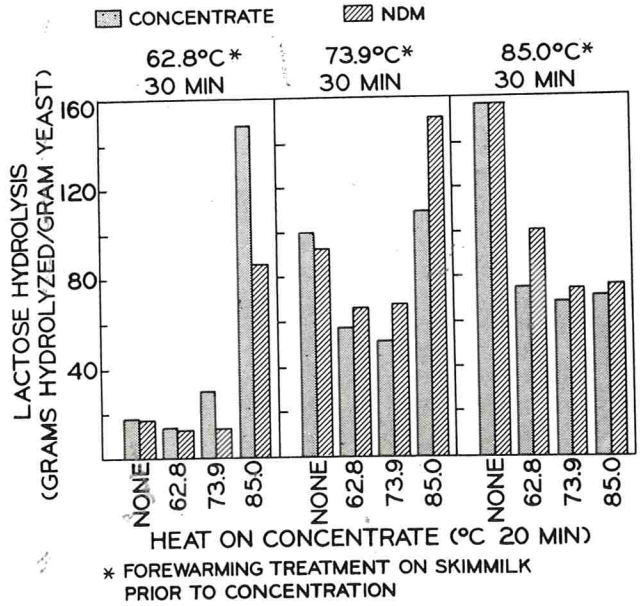

Figure 2. Effect of heating skimmilk concentrate on lactose hydrolysis.

trate samples. Monosaccharides were determined by the method of Tauber and Kleiner (7). The lactose hydrolysis results are reported as the number of grams of lactose hydrolyzed by the lactase in 1 g of dried yeast.

Results and Discussion

Figure 1 shows that lactose hydrolysis was greatly affected by the forewarming treatment of the skimmilk. With a high heat treatment, 85.0 C for 30 min, 156 g of lactose were hydrolyzed, whereas, with the low heat treatment, 62.8 C for 30 min, only 17 g of lactose were hydrolyzed. There were only minor differences between activities on the concentrated milk samples and the corresponding reconstituted NDM powders.

Results in Fig. 2 show that the hydrolysis was reduced in most of the concentrates and reconstituted NDM powders which had received an additional heat treatment just prior to spray drying. The concentrates heated at 85.0 C for 20 min, after forewarming at 62.8 and 73.9 C for 30 min, showed a substantial increase in lactose hydrolysis. These heat treatments exhibited a considerable difference between activities on the concentrate and reconstituted NDM. The commonly made observation that a high forewarming treatment tends to minimize viscosity changes caused by subsequent heat treatments also was noted in this experiment. The samples that were forewarmed at 62.8 and 73.9 C prior to concentrating and heating at 85.0 C formed heavy gels in the concentrate samples, whereas the sample forewarmed at 85.0 C did not. The high viscosity made the two former samples difficult to handle. This reduced the level of confidence in the results obtained with these two samples.

The reason for the great variations observed in lactose hydrolysis as a result of forewarming and subsequent heat treatments was not clearly evident from the results shown in Fig. 1 and 2. However, the results do emphasize the effect of the temperature history on lactose hydrolysis and the importance of knowing the temperature history of skimmilk if it is to be used as a substrate in the determination of lactase activity.

To aid in determining which fractions of skimmilk were primarily responsible for the variations in lactose hydrolysis in heated skimmilk, an additional series of experiments was made. Skimmilk, whey and a 5% lactose in salt solution were subjected to heat treatments of 62.8, 68.3, 73.9, 79.5, and 85.0 C. The salt solution was obtained by dialyzing skimmilk. From the results shown in Fig. 3, it is evident that heating lactose in the presence of milk protein had a great effect on lactose hydrolysis. Both whey and skimmilk gave increased activities at the higher heat treatments, whereas the lactose plus salt solution showed no variation. In the lactose plus salt solution, a white precipitate was formed after heating. This precipitate may have been Ca_3PO_4 and insoluble magnesium salts.

The complexity of the milk system makes it difficult to ascertain the role of milk proteins, but it seems evident that, through heating, the protein system can be altered to enhance lactose hydrolysis.

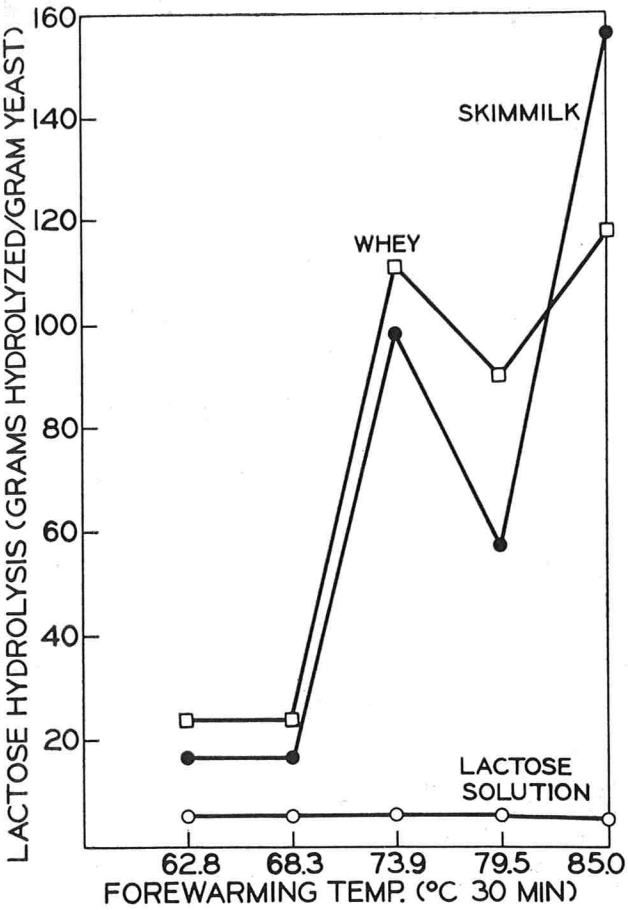

Figure 3. Effect of forewarming milk fractions on lactose hydrolysis.

Lactose hydrolysis of NDM powders appears to be inversely related to the protein reducing values reported by Swanson, et al. *(6)* who also worked with NDM powders that had been subjected to a variety of heat treatments. Whey proteins appear to have the greatest effect on decreasing lactose hydrolysis at forewarming temperatures of 62.8, 68.3, and 79.5 C. Casein appears to be responsible for the increased activity in skimmilk concentrate forewarmed at 85.0 C. From these data, it is not possible to conclude whether protein-protein interactions are completely responsible for the variation in lactose hydrolysis in heated milk products. More intensive studies must be made of the changes in the protein systems during the forewarming treatments and of the effect that these individual changes have on lactose hydrolysis.

Conclusions

The data obtained in this study suggests that changes occurring in the milk system during forewarming treatments have a major effect on the ability of yeast lactase to hydrolyze lactose in concentrated milk products. While more information is needed on the specific components affecting lactose hydrolysis in concentrated milk products, optimum conditions for use of the enzyme in these products are evident. For maximum lactose hydrolysis, skimmilk should be forewarmed at 85.0 C for 30 min. In food products where high heat NDM powders and high heat concentrates cannot be used, a larger concentration of lactase enzyme must be used to obtain comparable levels of lactose hydrolysis. Actual choice of conditions will depend upon the food product and the economics involved.

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EVALUATION OF CERTAIN MASTITIS DETECTION TESTS

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Abstract

A curvillinear regression resulted when the Feulgen-DNA test scores were plotted against the average concentration of leucocytes. Application of a logarithmic transformation to the average test data resulted in a linear correlation which slightly raised the correlation between the Feulgen-DNA Test and the leucocyte count. But, the application of a sample per cow transformation to the average test data from each of 30 representative cows, resulted in an approximate linear relationship and in significant correlations between the Feulgen, California, and Whiteside tests and the leucocyte count. Correlations in descending order were Feulgen> California> Modified Whiteside Test with respect to the leucocyte count.

The purpose of this report is to examine certain methods of evaluating mastitis detection tests based on the correlativity of a test with respect to the microscopic leucocyte count *(3)*.

In a previous report concerned with the evaluation of certain mastitis screening tests, correlation coefficients were calculated without concern for type of linearity involved in plotting a particular variable against the leucocyte count *(2, 3)*. This type of calculation, using raw data, assumes a linear relationship that may not obtain when qualitative are plotted against quantitative test results. Such a relationship was demonstrated by Paape, et al. *(5)*, in plotting the Feulgen-DNA and the Milk Quality Test (MQT) scores against the Somatic Cell Count.

Materials and Methods

This part of the report is for the purpose of verifying the work of Paape, et al. *(5)* and duplication of their treatment applied to NDSU data was attempted with the following exceptions:

(a) Milk samples were selected[1] at random from the University dairy herd and five commercial dairy herds so that there were 34 portions for each five Feulgen scores and 10 portions for the sixth score level.

(b) The Feulgen-DNA test was plotted versus the leucocyte count, modified *(4)*.

Correlation coefficients were calculated by IBM 1620 computer[1] and Daniels Batch Processing Stepwise Multiple Liner Regression Program.[2]

The range of the true mean was applied to the test variables by means of "student" distribution[3] and appropriate statistical calculations.

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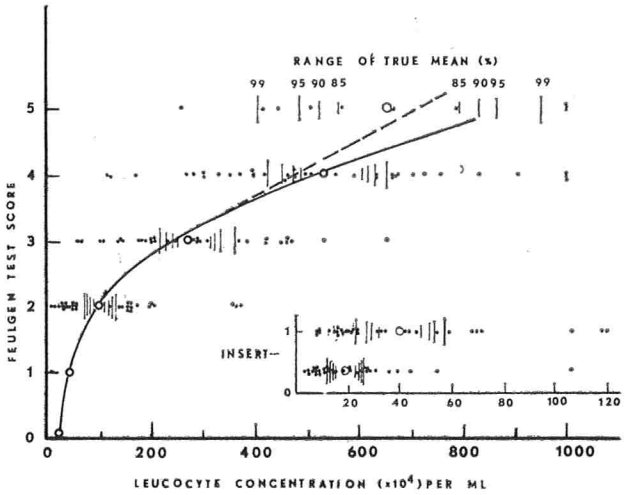

Figure 1. Feulgen mastitis test scores correlated with the concentrations of leucocytes resulting in a curvilinear relationship. Individual cell concentrations are indicated by ● and the average cell counts at each Feulgen score level by ◯. Bars at the 80, 90, 95, and 99% levels indicate the range of the true mean. Insert demonstrates the distribution on an enlarged scale.

Results and Discussion

The vertical bars, Fig. 1, show the range of the true mean at the 80, 90, 95, and 99% levels. The use of the student distribution[3] signifies that the true mean cell count will fall within the bars for each quality score reading 80, 90, 95, and 99% of the time, but it does not mean that the values will fall within these ranges at all times, since they represent only mean values.

The mean and standard deviations for leucocyte count x 10^4 at each Feulgen score level are tabulated as follows:

Feulgen-DNA score	0	1	2	3	4	5
Mean deviation	20	25	50	118	203	225
Standard deviation	16	37	58	145	246	269

Figure 1 demonstrates a curvilinear relationship between the Feulgen-DNA test scores and the concentration of leucocytes. Results are in substantial agreement with those reported by Paape, et al. *(5)*, with respect to the relationship of the somatic cell concentration to the Milk Quality Test and Feulgen-DNA Test scores. Paape, et al. *(5)*, performed a logarithmic transformation on the curvilinear data

[3]G. W. Snedecor, Statistical Methods, Fifth Edition. Iowa State College Press (Ames).

[4]Calculations available.

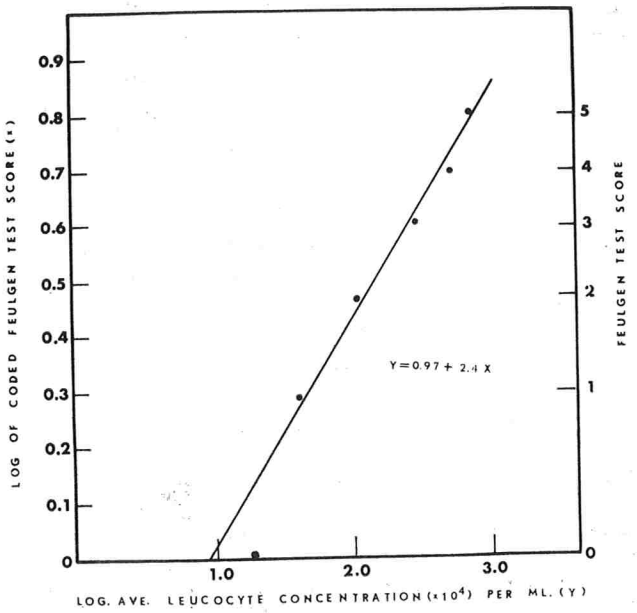

Figure 2. Application of a logarithmic transformation to the average mastitis test data.

which resulted in a linear relationship between the coded Feulgen-DNA score and the logarithms of the cell numbers, thus elevating the correlativity slightly over that of the score of the non-transformed data.

A similar transformation was applied to the NDSU average curvilinear data. The coded Feulgen score (score + 1), was correlated with respect to the logarithms of the numbers of leucocytes. A linear relationship resulted (Fig. 2) which is in general agreement with results reported by Paape, et al. (5).

The main criticism of this type of data transformation lies in reliance on the average microscopic cell count per Feulgen Test score, thus providing only one point per score and five points on a graph for both curvilinear and transformed data. It would seem that compilation of 4,945 samples in this manner might fail to represent true statistical validity.

One would expect that the mastitis test pattern of one cow to be distinctly different from that of other cows. This characteristic was observed in plotting the tests of samples from 30 individual cows against seasonal dates over a two year period.[5] This observation suggests that the application of a sample per cow transformation of mastitis test data might result in a closer relationship between the qualitative and the quantitative tests than with pooled data from two or more cows.

Thus a sample per cow tranformation was performed as follows: 30 cows were selected from the University herd, each with about 78 test readings over a period of 2 years. The pooled average readings from each of 30 cows were plotted, a linear relation was noted and the correlation coefficients of the quality test scores versus the leucocyte concentration were computed by the IBM 1620 computer[1] and Daniels Stepwise multiple linear regression program.[2]

For comparative purposes the average and composite correlations of University herd and the aver-

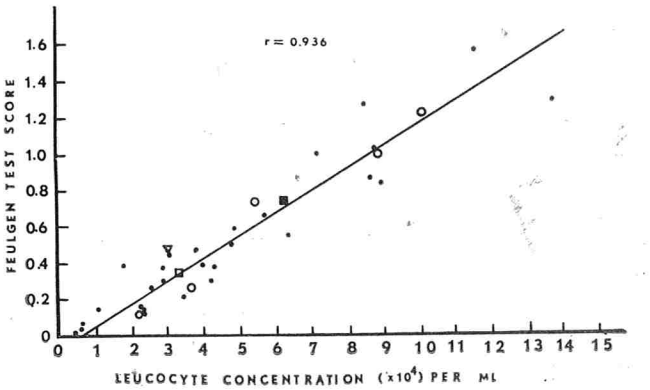

Figure 3. Sample per cow transformation; Feulgen - DNA versus Leucocyte Count ● average per cow samples 30 NDSU cows; ■ overall average 4,945 NDSU herd; Δ overall average NDSU composites; ◯ average per herd 5 commercial herds; □ overall average commercial herd samples.

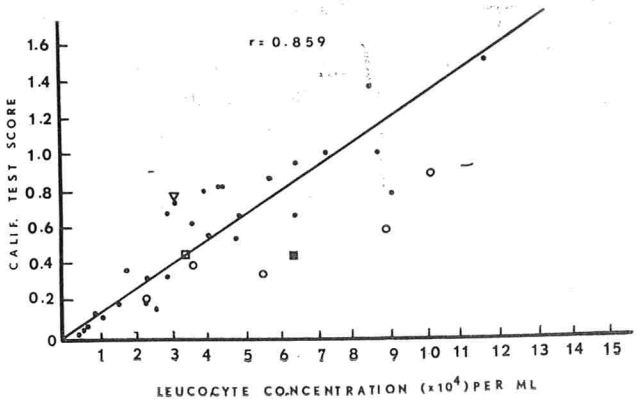

Figure 4. Sample per cow transformation; California Mastitis Test versus leucocyte count. Key is the same as in Fig. 3.

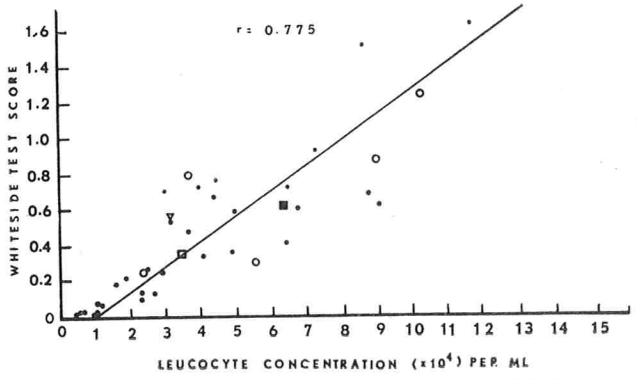

Figure 5. Sample per cow transformation; Modified Whiteside Test versus Count. Key is the same as in Fig. 3.

[5]From author's unpublished data.

TABLE 1. EFFECT OF DATA TREATMENT ON CORRELATION COEFFICIENTS OF CERTAIN MASTITIS TESTS VERSUS LEUCOCYTE COUNT[1]

Data treatment	Test correlated vs leucocyte count		
	Feulgen r	California r	Modified Whiteside r
Sample per cow			
Transformed	0.936	0.859	0.775
Untransformed	0.691	0.602	0.554
Difference	0.245	0.257	0.221

[1]Simple correlation coefficients of average (4,945) NDSU samples.

age values of 720 samples from five commercial herds also were plotted.

The pooled average scores from the Feulgen-DNA *(5)*, California *(7)*, and the Modified Whiteside test *(4)*, each were plotted against the average leucocyte concentration and results are demonstrated in Fig. 3, 4, and 5.

Results (Fig. 3, 4, and 5) demonstrate an approximate linear relationship of the qualitative mastitis tests to the leucocyte count, when a sample per cow transformation is applied to the curvilinear data. This transformation also takes into account variations among test readings from the University herd and from five commercial herds. In all instances, the check points fall close to the linear graph plotted against the average test readings from the 30 University cows employed in the analysis.

Correlation coefficients of tests evaluated (Fig. 3, 4, and 5) compared with corresponding correlations of the average untransformed NDSU herd test data are presented in Table 1.

Results show that in each instance the sample per cow transformation of average test data caused a definite elevation in correlations over the untransformed data.

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SURVIVAL OF *SALMONELLA TYPHIMURIUM* IN COLD-PACK CHEESE FOOD DURING REFRIGERATED STORAGE[1]

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Abstract

Fourteen batches of cold-pack cheese food were contaminated with *Salmonella typhimurium* during manufacture. Cheese food stored at 4.4 and 12.8 C was tested at weekly intervals and salmonellae were enumerated by means of a Most Probable Number technique. A rapid decline in number of salmonellae occurred during the first week of storage regardless of temperature or composition of the product. Survival beyond this time was more closely related to both conditions. Viable salmonellae could not be recovered, after 3 weeks at 12.8 C or 5 weeks at 4.4 C, from cheese food adjusted to pH 5.0 with lactic acid and fortified with 0.24% potassium sorbate. Substituting sodium propionate for sorbate resulted in 14 and 16 weeks of survival by salmonellae when cheese food was held at 12.8 and 4.4 C, respectively. Partial or complete replacement of lactic acid by acetic acid was accompanied by somewhat longer survival of salmonellae than when only lactic acid was used. Elimination of added acid from the cheese food resulted in survival of salmonellae for 6 and 7 weeks when potassium sorbate was present, for 16 and 19 weeks when sodium propionate was used, and in excess of 27 weeks when no preservative was added.

Salmonellae have been recognized as human pathogens for nearly 100 years. Salmonellosis is primarily an animal disease; human food-borne illness is a more or less accidental consequence of animal infection. Food-borne salmonellae are most often associated with poultry, poultry products, meat, and meat products. It is estimated that each year nearly two million Americans suffer from salmonellosis *(3, 7)*. Although the disease frequently is mild, approximately 100 persons in the United States die from salmonellosis each year and the annual economic loss attributed to this problem ranges from 10 to 100 million dollars *(13)*.

Milk once was a common vehicle for the dissemination of salmonellae. Since pasteurization has become common, milk has only rarely been incriminated in outbreaks of salmonellosis. However, nonfat dry milk has recently been associated with some outbreaks of salmonellosis caused by *Salmonella newbrunswick (9)*. Other salmonellae also have been recovered from this product since the onset, in 1966, of a vigorous testing program (9). During 1969 the following serotypes were found in samples of commercial nonfat dry milk: *Salmonella cubana, Salmonella minneapolis, Salmonella newington, Salmonella albany, Salmonella anatum, Salmonella tennessee,* and *Salmonella senftenberg (16)*.

According to a recent review by Marth *(9)*, outbreaks of salmonellosis attributable to cheese were reported as early as 1923 when, in Great Britain, persons contracted paratyphoid fever from consumption of contaminated cream cheese. Since then salmonellosis, nearly always caused by *Salmonella typhimurium* or *Salmonella typhi,* has been associated with Cheddar, Colby, Camembert, Romano Dolce, Teleme, Jack, Quarg, Cream, Cottage, and some other cheeses *(9)*. Persons handling milk used for cheese making or the milk itself most often served as sources of the salmonellae. In some instances cheese was consumed before it was aged sufficiently long to insure inactivation of salmonellae. Although cheese made from raw milk must be ripened at not less than 1.67 C (35 F) for at least 60 days prior to sale, there is ample evidence that this treatment will not eliminate viable salmonellae, if present, from cheese *(1, 5, 6, 12, 15)*. In the United States approximately 35% of the ripened cheese produced is used to manufacture processed cheese products of all types including cold-pack cheese food *(8)*.

Cold-pack cheese food is prepared from cheese, nonfat dry milk, dried whey, and some other ingredients. It is possible that the finished product could be contaminated with salmonellae by some of the ingredients. In addition to cheese, where extended survival of these organisms has been reported *(5, 6, 12)*, and nonfat dry milk, cheese food could be contaminated by dried whey which, on occasion has been found to contain salmonellae *(16)*. Contamination of a finished product with salmonellae from ingredients has been observed in other foods *(3)*. Additionally, cold-pack cheese food could be contaminated by personnel who are engaged in its production. Presence of salmonellae in this product

[1]Published with the approval of the Director of the Research Division, College of Agricultural and Life Sciences, University of Wisconsin.

TABLE 1. COMPOSITION OF EXPERIMENTAL BATCHES OF COLD-PACK CHEESE FOOD[1]

Ingredient	Experimental product						
	1	2	3	4	5	6	7
Cheddar cheese (g)	1698	1740	1740	1740	2112	1740	1740
NFDM (g)	100	100	100	100	125	100	100
Dried whey (g)	100	100	100	100	125	100	100
Butter (g)	76.2	76.2	76.2	76.2	95.1	76.2	76.2
Potassium sorbate (g)	5.4	—	5.4	5.4	6.8	—	—
Sodium propionate (g)	—	4.5	—	—	—	4.5	—
Lactic acid (ml)	8.5	10.5	7.0	—	—	—	—
Acetic acid (ml)	—	—	2.1	6.3	—	—	—
Water (ml)	241	261	261	261	327	261	261
Moisture (%)	41.81	43.36	43.65	43.73	43.58	43.49	43.24
Fat (%)	24.88	23.57	24.36	24.44	24.38	24.07	25.22

[1]Percentages of moisture and fat are average values obtained from tests on duplicate batches of each type of cheese food.

is of special significance because it receives no heat treatment nor is any additional aging required before it is consumed.

Since no information was available on the behavior of salmonellae in cold-pack cheese food, experiments were initiated to determine how long the organisms persist in such products when made according to different formulae and when stored at refrigeration temperatures. This paper reports results of the investigation. A preliminary report of this work has been given *(11)*.

MATERIALS AND METHODS

Culture

A 24 hr old nutrient broth culture of S. *typhimurium* (Department of Bacteriology, University of Wisconsin) was used to contaminate cold-pack cheese food during its manufacture. Sufficient culture was added to provide approximately 200 salmonellae per gram of product although this value was not attained in all experiments. The culture of S. *typhimurium* was maintained by daily transfer in nutrient broth.

Manufacture of cheese food and sampling procedure

The formulae used to prepare cheese food are given in Table 1. Two batches of cheese food were prepared according to each formula and data reported in this paper are average values obtained from tests on both similar batches of product. Cheddar cheese (6 to 10 months old) was ground using a hand-operated meat grinder after which the following ingredients were added: butter (melted in sterilized warm water prior to use), nonfat dry milk, dried whey, preservative (potassium sorbate or sodium propionate blended with dry ingredients before adding to the remainder of the product), and acid (lactic or acetic acid diluted with sterile water) when the pH of the product was adjusted. All ingredients were thoroughly mixed by hand (with sanitized rubber gloves in place) and with the aid of a potato ricer. Finally S. *typhimurium* (diluted with sterile buffered water just prior to use) was added and the mixing process just described was repeated. Preliminary trials with *Serratia marcescens* demonstrated that the mixing process was adequate to insure uniform distribution of added bacteria in the cheese food. Cheese food was then filled into 2 oz plastic containers fitted with screw caps and was stored at 4.4 and 12.8 C. The product was tested initially for moisture and fat contents, pH, and number of viable salmonellae. Stored cheese food was examined at weekly intervals for its pH value and number of viable salmonellae.

Federal standards for cold-pack cheese food require that the product contains a minimum of 23% butterfat and a maximum of 44% moisture. Additionally, use of 0.20% sorbic acid or 0.30% sodium propionate is permitted. All cheese food prepared in these trials complied with the indicated standards.

Fat, moisture, and pH determinations

The fat content of cheese food was determined by the modified Babcock procedure as described by Van Slyke and Price *(17)*. Moisture in the product was determined by drying 3 g cheese food in a 50 ml beaker at 110 C for 16 hr in a forced draft oven. The pH value was measured with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer.

Enumeration of salmonellae

Salmonellae in cheese food were enumerated by means of the Most Probable Number (MPN) technique. Each value for a given batch of cheese represents the average of results obtained by testing three samples. Consequently, each value reported in the figures represents the average of 6 samples, three from each of two batches of cheese food. Twenty grams of cheese food was blended (4 min in a Waring blendor) with 180 ml of a sterile 2% sodium citrate solution previously cooled to 5 C. Subsequent dilutions were made with the sodium citrate solution.

After blending, 1 ml aliquots of appropriate dilutions were transferred to tubes each of which contained 9 ml of brain heart infusion broth (Difco). After overnight incubation at 37 C, 1 ml quantities were transferred to tubes containing 9 ml each of selenite-cystine broth (Difco). These cultures were incubated at 37 C for 24 hr and a sample from each tube was then streaked on SS agar (Difco) modified by adding 10 g sucrose (to screen for sucrose fermenting organisms) and 6.5 g agar (to facilitate streaking) per liter. When used as described, SS agar recovered essentially only salmonellae from cheese food. This, perhaps, is more readily understood when it is realized the 6-10 month old Cheddar cheese and the other ingredients used are not likely to contain many

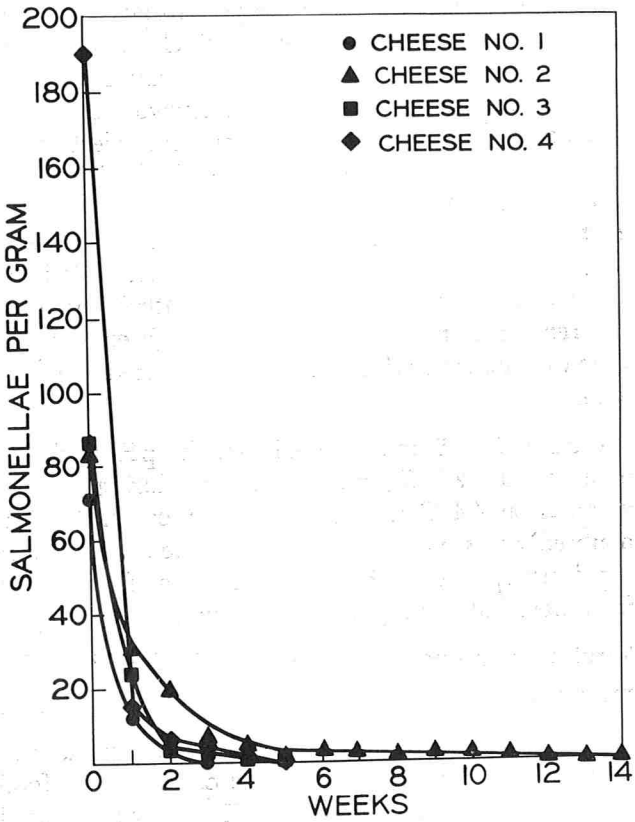

Figure 1. Survival of *Salmonella typhimurium* in cold-pack cheese food adjusted to pH 5.0, fortified with preservative, and stored at 12.8 C. Details on composition of each cheese food are in Table 1.

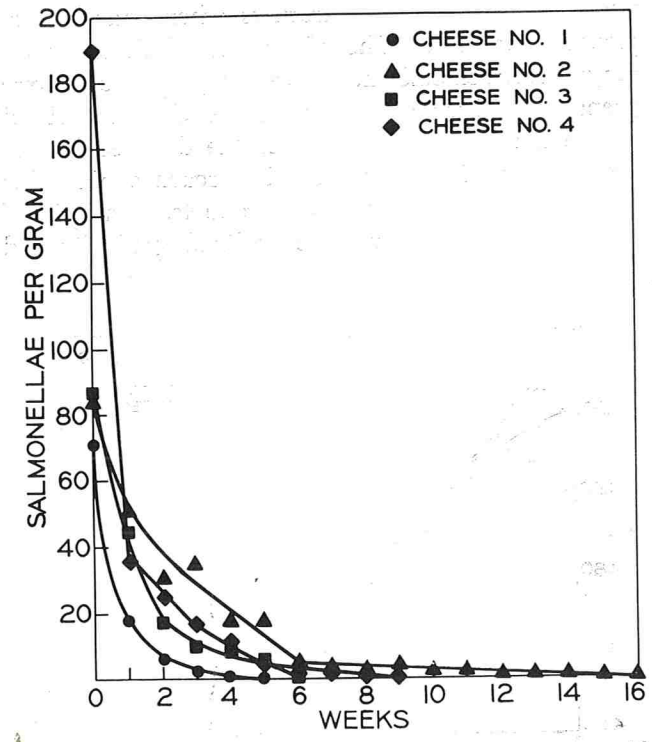

Figure 2. Survival of *Salmonella typhimurium* in cold-pack cheese food adjusted to pH 5.0, fortified with preservative, and stored at 4.4 C. Details on composition of each cheese food are in Table 1.

of the organisms which normally cause difficulty in recovery of salmonellae. Plates were examined after 24 hr at 37 C and typical *Salmonella* colonies were streaked onto and stabbed into triple sugar iron agar slants. These slants were incubated 24 hr at 37 C and checked to insure that the reaction was typical of that produced by salmonellae.

RESULTS AND DISCUSSION

Survival of salmonellae in cheese food with added acid and preservative

Figures 1 and 2 present data on the survival of salmonellae in cold-pack cheese food adjusted to pH 5.0 with lactic and/or acetic acid and fortified with either potassium sorbate or sodium propionate. Data on the pH values of these products during storage are recorded in Fig. 5 and 6.

It is evident that the number of viable salmonellae in all four types of cheese food declined most rapidly during the first week of storage at both temperatures (4.4 and 12.8 C). A less precipitous decline continued during subsequent weeks of storage. Salmonellae in cheese food containing both lactic acid and potassium sorbate declined to nondetectable levels in 3 and 5 weeks when the product was held at 12.8 and 4.4 C, respectively. In contrast, 14 and 16 weeks at the same temperatures were required before salmonellae could not be recovered from cheese food made with sodium propionate instead of potassium sorbate.

Although potassium sorbate is commonly thought to be more inhibitory to yeasts and molds than is sodium propionate *(10)*, Doell *(2)* reported that the chemical at a concentration of 0.1% and at pH values of 5.0 and 6.0 was bacteriostatic to some salmonellae. A bactericidal effect was not noted under the same conditions during a 48 hr incubation period. Doell did observe inactivation of salmonellae in 24 or 48 hr at pH 5.0 when the concentration of sorbate was increased to 1.0%. Extended storage in these experiments may have accomplished what the increase in concentration did in the tests by Doell.

Replacement of lactic acid, completely or in part, with acetic acid failed to enhance destruction of salmonellae in cheese food. In fact, the organisms persisted for 4 and 5 weeks at 12.8 C and for 6 and 9 weeks at 4.4 C. The longer periods of survival just indicated were noted when acetic acid only was used and the shorter times when a mixture of lactic and acetic acid was added. Even though acetic acid apparently had no beneficial effect over lactic acid, salmonellae in cheese foods containing acetic acid and sorbate (No. 3 and 4) remained viable for approximately one-third to one-half as long as those in cheese which contained lactic acid and sodium propionate.

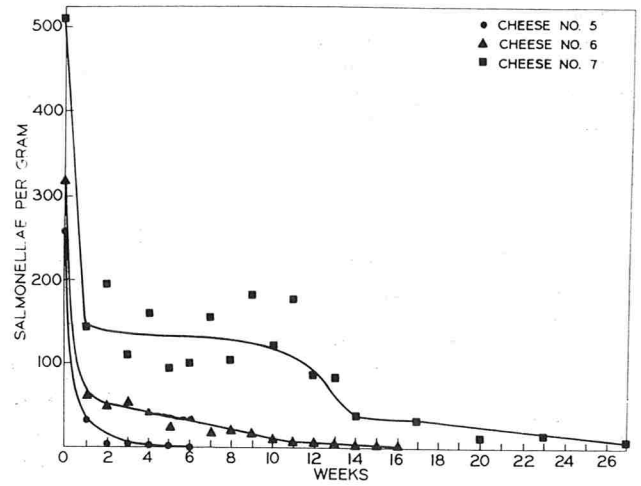

Figure 3. Survival of *Salmonella typhimurium* during storage at 12.8 C in cold-pack cheese food made without pH adjustment and with and without preservative. Details on composition of each cheese food are in Table 1.

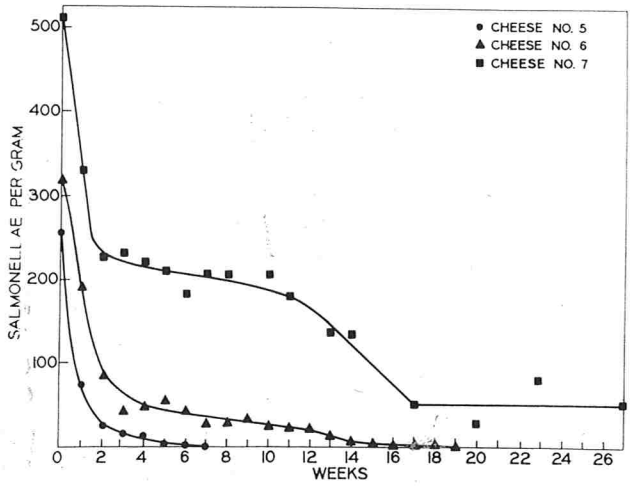

Figure. 4. Survival of *Salmonella typhimurium* during storage at 4.4 C in cold-pack cheese food made without pH adjustment and with and without preservative. Details on composition of each cheese food are in Table 1.

Several reports *(4, 9, 14, 18)* have indicated that organic acids are inhibitory to salmonellae. Propionic and acetic acids are usually thought to be more active than citric and lactic acids. Recently Subramanian and Marth *(14)* observed that citric acid retarded growth of *S. typhimurium* more than did lactic or hydrochloric acids. Wethington and Fabian *(18)* noted that *Salmonella schottmuelleri, S. typhimurium, Salmonella paratyphi, Salmonella enteritidis, Salmonella choleraesius,* and *Salmonella pullorum* survived for 144, 144, 156, 156, 156, and 132 hr, respectively, in mayonnaise made to contain 0.15% acetic acid (pH 5.0) and held at room temperature. Goepfert et al. *(5)* reported that the presence of 0.1% acetate in skimmilk at pH 4.9 enhanced inactivation of *S. typhimurium* and they suggested that accumulation of this acid during ripening of Cheddar cheese might contribute to the demise of salmonellae in cheese. In contrast to this, Hargrove et al. *(6)* claimed that acetic acid had no apparent effect greater than that of other acids on survival of salmonellae in Cheddar cheese made by direct acidification. Differences in the effect of acetic acid on salmonellae, including those reported in this paper, noted by various investigators are probably attributable to different environmental conditions which existed and which concurrent with acetic acid, affected the bacteria.

Data in Fig. 5 and 6 reveal that the pH of cheese food held at 12.8 C tended to decline during storage, whereas, at 4.4 C it remained rather constant. Undoubtedly this change in pH contributed to the more rapid disappearance of viable salmonellae from cheese food stored at the higher temperature.

Survival of salmonellae in cheese food with added preservative

Figures 3 and 4 provide data on the disappearance of viable salmonellae from cold-pack cheese food made without adjusting the pH but with added potassium sorbate (No. 5) or sodium propionate (No. 6). Detectable salmonellae persisted for 6 and 7 weeks at 12.8 and 4.4 C, respectively, when cheese food contained potassium sorbate and for 16 and 19 weeks at the same temperatures when sodium propionate was used. The pH values of cheese foods ranged between 5.20 and 5.26 when the products were prepared. During storage at 4.4 C, the pH of cheese food with potassium sorbate dropped by approximately 0.1 unit but failed to reach a pH value of 5.0 as existed in acidified products. The pH of the product with propionate declined gradually and

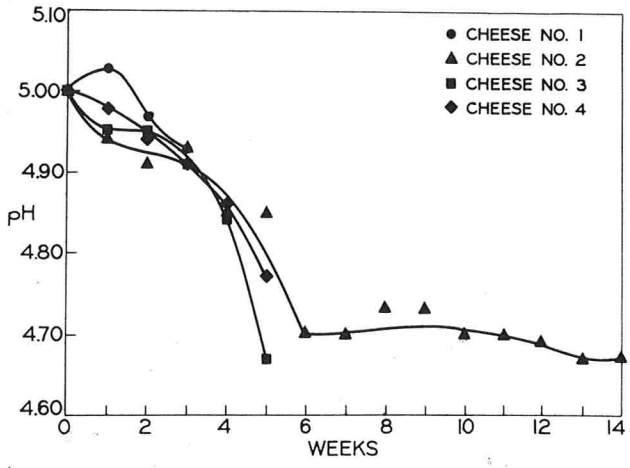

Figure 5. The pH values of acidified cold-pack cheese food containing preservatives and stored at 12.8 C.

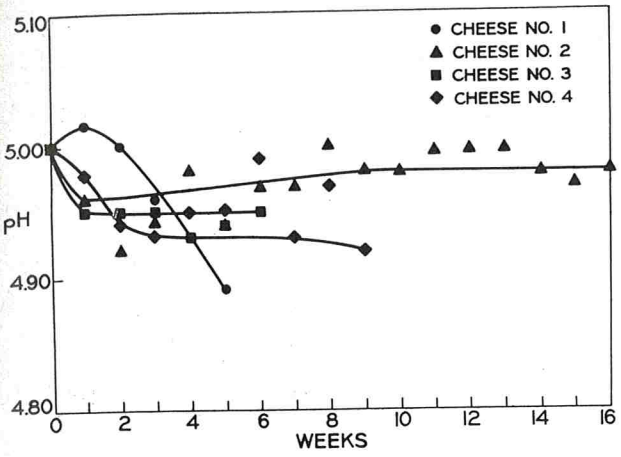

Figure 6. The pH values of acidified cold-pack cheese food containing preservatives and stored at 4.4 C.

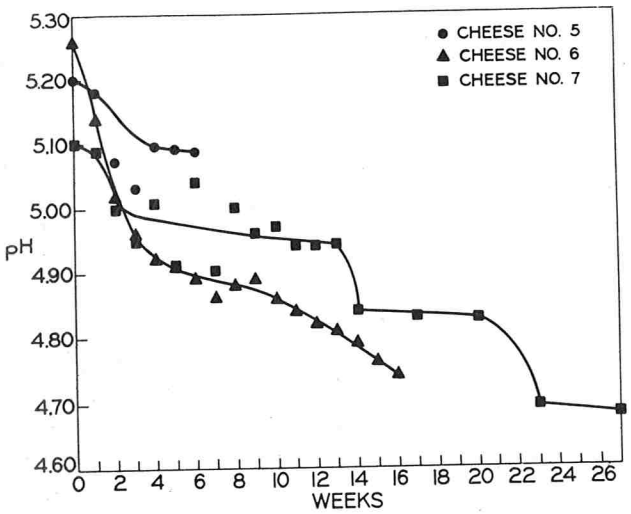

Figure 7. The pH values of cold-pack cheese food made without added acid, with or without preservatives, and stored at 12.8 C.

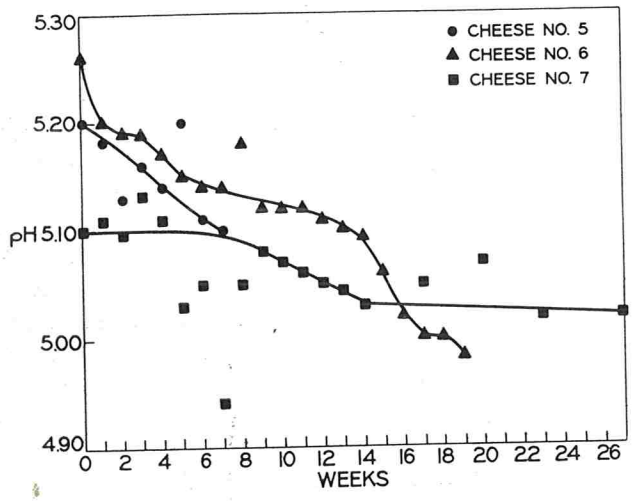

Figure 8. The pH values of cold-pack cheese food made without added acid, with or without preservative, and stored at 4.4 C.

a value of 5.0 was attained after 17 weeks of storage. Undoubtedly the higher pH of cheese food contributed to extended survival of salmonellae in the product held at 4.4 C. The decline in pH was greater in cheese food held at 12.8 C and this is probably related to more rapid demise of salmonellae from the product.

Survival of salmonellae in cheese food free of added acid or preservative

Data in Fig. 3 and 4 (No. 7) indicate that viable salmonellae remained in unfortified cheese food after 27 weeks of storage at either 12.8 or 4.4 C. A somewhat higher population appeared in cheese food stored at the lower temperature. The pH value of the product held at 4.4 C remained above 5.0 throughout storage, whereas cheese food at 12.8 C attained a pH value of 5.0 after two weeks of holding and then continued to decline below 4.7 after 23 weeks. In spite of this drop in pH, viable salmonellae persisted for at least 27 weeks. This suggests that in a product as complex as cheese food, pH (within the range associated with the product) is only one factor which governs survival of salmonellae.

Observations made in these experiments emphasize that cold-pack cheese food must be prepared from high quality ingredients which are free of hazardous microorganisms and the product must be handled to preclude contamination by such microorganisms during its manufacture. If present, salmonellae could survive in the product until it reaches the consumer, particularly if the pH of cheese food is not adjusted and if potassium sorbate is not used.

ACKNOWLEDGMENT

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KENTUCKY HAULERS TOLD THEY ARE KEY TO MILK QUALITY

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"Picking up milk from farms which do not have valid permits is a serious violation which occasionally plagues the bulk milk hauling industry," commented Gayle Shrader, Chief of Milk Control, Louisville and Jefferson County Department of Public Health, Louisville, Kentucky. Speaking to 150 haulers at meetings in Elizabethtown, Kentucky, Shelbyville, Kentucky and Palmyra, Indiana, Shrader urged them to eliminate partial pickups, reject milk with known added water, to rinse producers' tanks after pickups, and to take samples in the proper manner.

"Haulers are an important cog in keeping the milk industry alive," said Dudley Conner, Supervisor of the Milk Control Program, Kentucky State Health Department. Conner challenged the haulers to reject milk that does not meet state health department regulations. "An Interstate Milk Shippers rating below 90 prevents milk from being moved into another state," he reminded the hauler delegates.

A. P. Bell, Director of the Division of Environmental Services for the Louisville-Jefferson County Health Department, emphasized that there is no way to improve milk that has undergone bacterial and chemical degradation.

"You aren't doing the producer a favor by taking a partial load of milk," said featured speaker Joe Johnson, of Arlington, Texas. "After all, the producer is paying the hauling bills and deserves a fair shake," continued the AMPI representative. Johnson urged the Kentucky milk transporters to agitate the milk properly before taking samples and to carry a standard thermometer to check the bulk tank temperatures. "Too often," he said, "the tank thermometer is not functioning properly."

"Quality is our business because quality keeps us in business," stated Kentucky hauler Joe Monin of Monin Brothers Transfer. Other haulers who participated on the training programs were Cleo Mull, Mull's Milk Transfer, and Mr. Robert Hamilton, Hamilton's Milk Service.

Plant processor views on the role of the hauler were given by Mr. Ed Napier, Sealtest Foods, Louisville, Kentucky, and Mr. Jim Spillman, Dairymen, Inc. – Kyana Division, Louisville, Kentucky. Jim McDowell, field supervisor for Dairymen, Inc. – Kyana Division, spoke on hauler-fieldmen teamwork.

In summarizing the meetings, Dr. C. Bronson Lane of the University of Kentucky Animal Sciences Department said that an effective hauler is totally involved, projects a good image, and is completely informed about his role as a team leader in obtaining quality milk.

The Louisville area bulk milk hauler training programs were sponsored by the state and local health departments of Kentucky, the U. K. Cooperative Extension Service, and the dairy industry of Louisville.

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STUDIES USING THE DIRECT MICROSCOPIC SOMATIC CELL COUNT

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ABSTRACT

Factors which might contribute to variance of cell estimates in milk, using the Direct Microscopic Somatic Cell Count (DMSCC) were investigated. Comparisons of cell estimates obtained from counting cells in nine areas of 20 circular milk films produced no evidence of uneven distribution of cells on these films. Estimates of cells by DMSCC were not affected by the temperature of the milk, or the method of spreading, when milk films were prepared. Standard deviations and means of counts indicated no significant difference in cell estimates obtained by three operators. Cell estimates from counts made in horizontal and vertical diameter strips of milk films showed no significant differences among means or variances relating to strip direction. Cells were counted in diameter strips (as defined by a wide and narrow ocular reticle) at two magnifications (450 x and 1,000 x). Cell estimates based on counts from the wide strips had lower standard deviations than those from the narrow strips.

Confidence limits were established for estimating 1 x 10^6 cells per ml of milk at p = 0.05.

A procedure for determining precision within laboratories and permitting a comparison between laboratories is presented.

The Direct Microscopic Somatic Cell Count (DMSCC) *(6)*, was developed by the Sub-Committee on Screening Tests of the National Mastitis Council to improve the accuracy and precision of the Direct Microscopic Leukocyte Count *(2)*. The area examined using the DMSCC is described as four diameter strips on two circular milk films. Expected confidence limits for the DMSCC have been determined *(6)*.

This study was initiated to determine factors that might contribute to variance of cell estimates using the DMSCC. Some factors which might influence cell estimates that were included in this study were: *(a)* distribution of cells over the surface of a film of milk, *(b)* effect of temperature of milk and method of spreading milk film, *(c)* variation between operators, *(d)* variation between direction of strips on milk films (horizontal and vertical), *(e)* effect of width of strip, and *(f)* effect of two magnifications (1,000 x and 450 x).

MATERIALS AND METHODS

Preparation of milk films

The milk films in this study were prepared as described for the DMSCC *(6)*, except that the slides contained 3 rows of 5 circular 1 cm^2 areas surrounded by etching[1].

Microscope

One ocular (wide field) of the binocular microscope[2] was equipped with a reticle with two sets of parallel lines[3] which permitted the examination of a portion of the milk film of known width as defined by the parallel boundaries in the microscope field. The band widths were 0.078 mm (wide band) and 0.026 mm (narrow band) using 1000 x magnification; and 0.179 mm and 0.058 mm using 450 x magnification. Horizontal and vertical diameter strips of the milk films were located as described elsewhere *(6)*.

Examination for distribution of cells on milk film

Eleven horizontal and 11 vertical strips were examined (1000 x magnification, wide reticle band) on each of 20 circular milk films. Following the location of the horizontal diameter strip, 5 additional strips on each side of and parrallel to the horizontal strip were examined. All strips were located with 1 mm distance between centers of adjacent strips. A similar pattern was followed for the location of 11 vertical strips. The relative location of 22 strips that were examined on each film is depicted in Fig. 1. The vernier scale on the mechanical stage permitted the location of all strips that were not diameter strips.

It was necessary to develop a mathematical equation for determining the area of each of the strips. In a circle whose area is 1 cm^2, the radius is approximately 5.6419 mm.

To determine the area of a parallel sided strip any place on the circle, the following equations were derived:

$$\text{(I)}\quad A(h) = h\sqrt{1 - h^2} + \arcsin h$$

$$\text{(II)}\quad A = 0.5 \sin 2\ominus + \ominus$$

These equations permit the determination of the area A between a diameter and a lesser chord, in this instance, one of the boundaries of a parallel sided strip (Fig. 2), where:

(a) The radius of circle is 1.
(b) h is expressed as a portion of the radius.
(c) $\sin \ominus = h$, and $\ominus$ (arcsin h) is expressed in radian measure.

Let H_1 represent the distance in millimeters from the diameter to the nearer side of a strip, and H_2 represent the distance in millimeters from the diameter to the farther side of the strip (Fig. 2, B). Solve first for the area determined by using H_1, then that area determined by using H_2. It is necessary to convert the actual H values in millimeters into

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[1]George T. Walker, 2218 University Avenue, Minneapolis, Minnesota 55414.

[2]American Optical Co., Eggert and Sugar Road, Buffalo, N. Y. 14215.

[3]Special reticle developed for SubCommittee of National Mastitis Council Research Committee.

units of h which fall between 0 and 1. To accomplish this:

$$(III)\quad h = \frac{H \text{ mm}}{5.6419 \text{ mm}}$$

The steps necessary for solution of equation (II):

(a) Determine H_1 in mm from the vernier scale on the mechanical stage of the microscope (Note: The difference between H_1 and H_2 was 0.078 mm in each instance with the reticle-microscope combination used.)

(b) Convert H to h using equation (III).

(c) From a table of natural functions for angles expressed in radians, determine a value for $\ominus$, which is the arcsin h.

(d) Double $\ominus$.

(e) From the table (step 3), determine the sin of 2 $\ominus$.

(f) Divide this value (step 5), by 2.

(g) Add the value $\ominus$ and 0.5 sin 2 $\ominus$.

(h) Divide this value (step 7) by 3.14159 to determine the portion of the area of the circle between a diameter and the nearer side of the strip.

(i) Solve the equation again using H_2 value (steps 1 through 8).

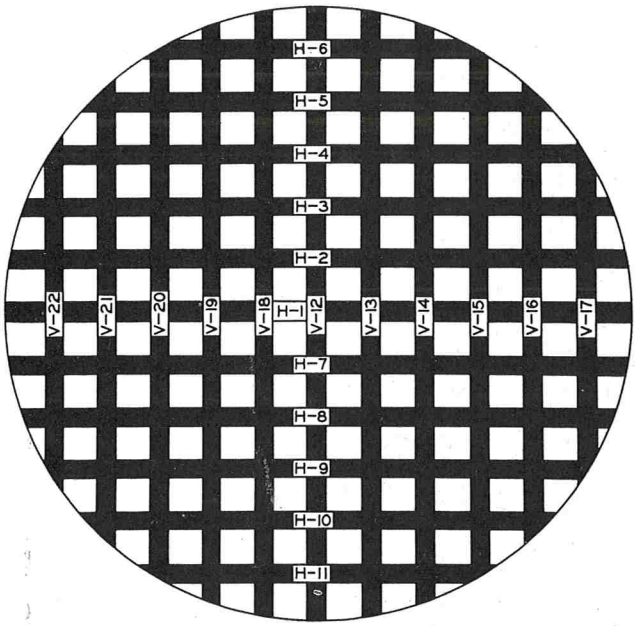
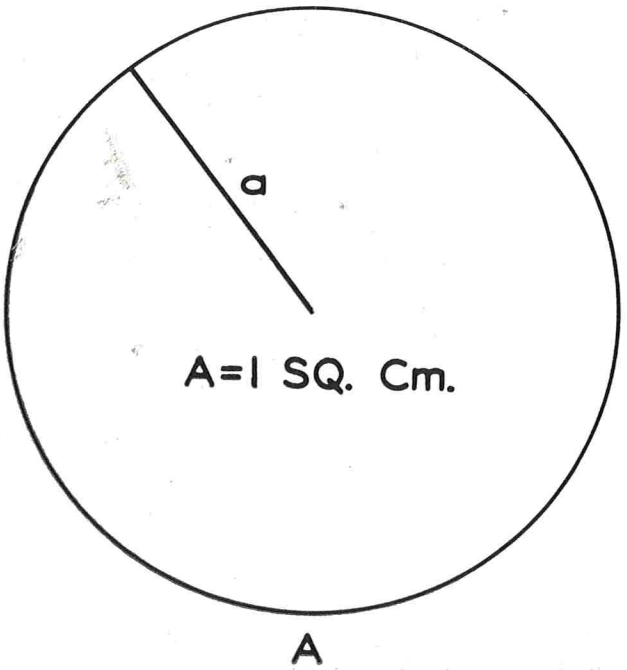
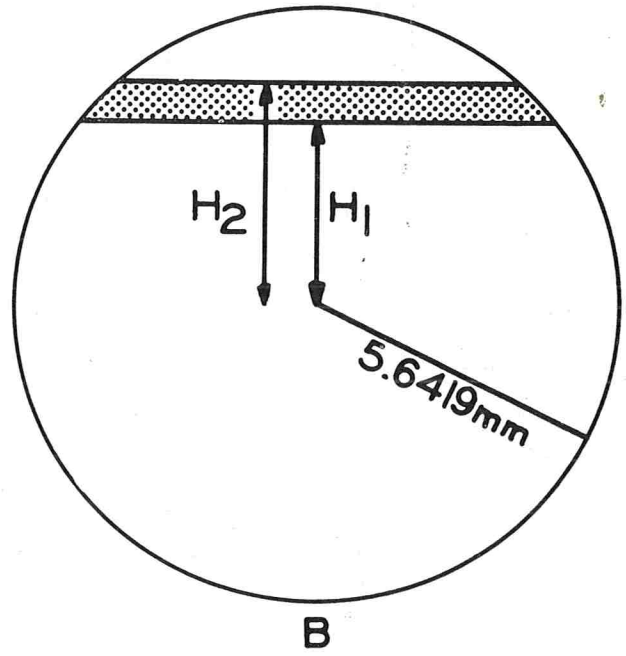


Figure 1. Diagramtic representation of relative location of 22 strips examined for distribution of cells on film of milk.





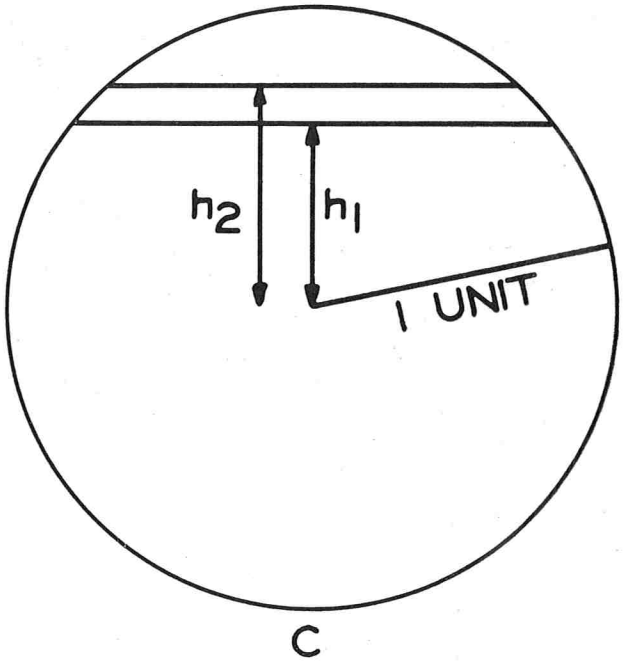


Figure 2. Figures used to explain the computation of the area of a strip of circular milk film.

(i) Subtract the value determined by using H_1 from the value determined by using H_2. This difference represents the portion of the area of the circle which falls within the strip under consideration.

It is of academic interest to note that a diameter strip, whose width is 0.078 mm with ends that are arcs of a circle with an area of 1 cm^2 has an area of 0.88012 mm^2, whereas, if the strip were considered to be a true rectangle 0.078 mm x 11.2838 mm, the area would be 0.880136 mm^2. Therefore, for practical purposes, the diameter strips described in this study could be considered rectangles.

Milk Samples

Fresh, bulk milk samples were obtained from Madison Milk Producers, Madison, Wisconsin. Films were prepared the same day that samples were obtained.

RESULTS

Distribution of cells on film of milk

Trial I. Ten milk samples with various concentrations of cells (150,000 to 3,000,000 cells/ml) were used. Strips were numbered from 1 to 22 (Fig. 1). Cell estimates were derived from 9 areas of each film using various combinations of strips, (Table 1). Analysis of variance indicated no significant difference among 9 estimates of cell counts within each film.

Trial II. Ten replicate films from one milk sample were used (approximately 400,000 cells/ml). Cell estimates were derived from 9 areas of each film using various combinations of strips as in trial I (Table 1). Analysis of variance indicated no significant differences within or between estimates from ten films.

Temperature and method of spreading milk

In order to determine if the temperature of milk or the method of spreading milk affected cell estimates, the following trial was conducted. Milk from five different bulk milk samples (range–600,000 to 1,500,000 cells/ml) were used to make films. One series was made with cold milk (4 C) using a metal rod to spread the milk. A second series was made from cold milk (4 C) using the pipette (0.01 ml) for spreading the milk. A third and fourth series were made from warm milk (35 C) and the milk was spread with a metal rod and the pipette as above. A 2 x 2 factorial analysis of variance indicated no significant difference between methods and no interaction between methods.

Operators

The analysis of variance comparing cell counts by three operators on 576 milk samples (Table 2) revealed that there was no significant difference between group means. The standard deviation of each of the three operators was similar.

Horizontal and vertical strips

The analysis of variance comparing cell estimates from counts in horizontal and vertical strips (Table 3) indicated that there was no significant difference in the means of either direction. The standard deviation from strips of each direction was similar.

Strip width and magnification

A summary of the results comparing strip width and magnification are presented (Table 4). A paired t test was used to compare the variance and means of each method. There was no significant difference between means or variance using either 450 x or 1000 x magnification. Also, there was no significant difference between the means obtained using wide or narrow strips. However, there was significantly more variation ($p = 0.001$) in cell estimates obtained using narrow strips than in cell estimates obtained using wide strips.

CONFIDENCE LIMITS

The components of variance of the remaining factors were computed using only data from wide strip, 1000 x magnification (Table 5). When the various components were separated, they were as follows:

Estimated residual variance	126
Estimated sub-sample variance	40
Estimated film variance	25

Using 1,000,000 cells/ml as the nominal rejection level for milk, 95% confidence limits were computed (Table 6). Confidence limits are expressed in cell numbers.

DISCUSSION

Precision within laboratories and standardization between laboratories

The ideal reticle as described for use in the DMSCC for the purpose of identifying milks that contain 1,000,000 or more cells per milliliter, will define a diameter strip on the milk film which contains approximately 100 cells. Microscopes equipped with different oculars (eg: wide field, huygenian, 10 x, 7.5 x, etc.) may require reticles with different dimensions in order to achieve a strip width that will include 100 cells *(6)*.

Comparison of cell counts between laboratories has little meaning unless something is known of the level of precision of the DMSCC in each laboratory. If confidence limits for DMSCC were developed for each laboratory and were of similar value, then meaningful comparison between laboratories could occur. Confidence limits would also give an indication of agreement between any laboratory and the guide lines suggested for the DMSCC *(6)*.

The following is a description of a procedure from which confidence limits for the DMSCC may be developed:

TABLE 1. DESCRIPTION AND IDENTIFICATION OF NINE AREAS OF MILK FILM WITH MICROSCOPE WORKING FACTORS AND MEANS OF CELL ESTIMATES

Area Examined	Strips	Working factor	Means x 10^{-6} Trial I	Trial II
Center horizontal strip	H-1	11,362	0.995	0.442
Center vertical strip	V-12	11,362	1.026	0.442
Center vertical and horizontal strips	H-1, V-12	5,681	1.011	0.442
All horizontal strips	H-1 through H-11	1,278	0.971	0.434
All vertical strips	V-12 through V-22	1,278	0.987	0.436
All strips	H-1 through V-22	639	0.979	0.435
Outer area strips	H-5, H-6, H-10, H-11, V-16, V-17, V-21, V-22	2,431	0.922	0.462
Intermediate area strips	H-3, H-4, H-8, H-9, V-14, V-15, V-19, V-20			
Center area strip (3 horizontal and 3 vertical strips)	H-1, H-2, H-7, V-12, V-13, V-18	1,585 1,912	0.984 1.009	0.421 0.430

TABLE 2. COMPARISON OF CELL COUNTS (DMSCC) FOR THREE OPERATORS AND ANOVA TABLE

(Cell estimates = x 10^{-4})

Operator	Number of observations	Group mean	Group standard deviation
1	576	116.1	58.7
2	576	119.5	60.6
3	576	118.5	56.9

Analysis of Variance Table

Source	Degrees of freedom	Mean square
Between groups	2	1,814.2
Within groups	1,725	3,451.5
F ratio = 0.526 n.s.		

TABLE 3. COMPARISON OF CELL ESTIMATES (DMSCC) FROM COUNTS MADE IN HORIZONTAL AND VERTICAL STRIPS

(Cell estimates = x 10^{-4})

Direction	Number of observations	Group mean	Group standard deviation
Horizontal	288	111.3	55.4
Vertical	288	111.5	53.7

Analysis of Variance Table

Source	Degrees of freedom	Mean square
Between groups	1	5.35
Within groups	574	2,977.72
F ratio = .002		

A fresh milk sample is selected that contains approximately the cell concentration that is to be used as a rejection level for acceptable milks (example: 1 or 1.5 million). The cell count in the sample chosen may be approximated by a single microscope count. Use of indirect tests should be avoided for the selection of this sample.

This sample should be used to make 20 films. Each film should be examined across one diameter either vertical or horizontal according to the operator's preference. Variation (S^2) is determined using a standard method (*5*, p. 58). Example: *(a)* Determine mean (average of 20 counts). *(b)* Subtract each of the counts from the mean. *(c)* Square each of these values. *(d)* Add these values. *(e)* Divide this sum by 19. Confidence limits are obtained by using this equation (*5*, p. 58):

$$\frac{2S^2}{\sqrt{n}} = L$$

L = 95% confidence limits
n = number of films examined

Let us assume that $S^2 = 10$. Then, for n = 1, the confidence limits are ±20. Therefore, assuming the microscope working factor is 10,000 and the rejection level is 1,000,000 cells/ml, it would be necessary to obtain a count of 120 cells on a single strip in order to be confident that the original sample contained 1 million or more cells per milliliter. Similarly, a count of 80 or less per strip would be necessary to know with 95% confidence that the sample had less than 1 million cells per milliliter. The confidence limits would be reduced in the following manner: for two srtips, L = ± 14.1; for three strips, L = ± 11.5; for four strips, L = ± 10, etc.

The milk sample may be sent to another laboratory where four films should be prepared and counted across one diameter each. The average of these four counts should fall within the confidence limits of four strips as computed above. This is necessary to confirm the operator's ability to pipette 0.01 ml milk accurately and determine if cell identification was performed correctly.

TABLE 4. COMPARISON OF MEAN CELL ESTIMATES AND STANDARD DEVIATOINS OF TWO STRIP WIDTHS AND TWO MAGNIFICATIONS

Band	Width	Magnification	Strip working factor	Number of observations	Group mean	Group standard deviation
Wide	0.179 mm	450 x	4,950	80	66.4	9.20
	0.078 mm	1,000 x	11,362	80	65.8	9.67
Narrow	0.058 mm	450 x	15,384	80	69.7	12.15
	0.026 mm	1,000 x	33,333	80	62.4	13.17

TABLE 5. COMPONENTS OF VARIANCE FOR BULK, SUB-SAMPLE, AND FILMS

Sources	Degree of freedom	Mean square	Expected mean square
Bulk	3	541,889	$\frac{\sigma^2}{e} + 48\frac{\sigma^2}{B} + 4\frac{\sigma^2}{S} + 2\frac{\sigma^2}{F}$
Sub-sample	44	335	$\frac{\sigma^2}{e} + 4\frac{\sigma^2}{S} + 2\frac{\sigma^2}{F}$
Film	48	175	$\frac{\sigma^2}{e} + 2\frac{\sigma^2}{F}$
Residual	480	125	$\frac{\sigma^2}{e}$

TABLE 6. 95% CONFIDENCE LIMITS FOR MEAN CELL COUNTS OF 100 CELLS PER STRIP ASSUMING THE MICROSCOPE WORKING FACTOR IS 10,000.

Area of film(s) examined to determine cell estimates	Cells per strip			
	12 sub-samples	4 sub-samples	2 sub-samples	1 sample
One strip on one film	±6.5	±11.3	±15.7	±22.6
Two strips on one film	±5.3	± 9.3	±13.1	±18.5
One strip on each of two films	±5.1	± 8.1	±12.4	±17.6
Two strips on each of two films	±4.3	± 7.5	±10.6	±15.0

By use of this method, laboratories could accept or reject milk with a 95% assurance that results of the DMSCC are comparable between different laboratories.

Results from this experiment reinforce the earlier report *(6)* of the possibility of repeatability of the DMSCC among different operators. Since no effects on cell counts were determined from temperature of milk or method of application of milk at the time films are prepared, there is no reason to vary from the standard procedure of the DMSCC. This investigation also reinforces the report *(6)* that no difference should be expected in cell numbers, related to direction of counting in strips (horizontal or vertical).

Unlike the distribution of cells that has been reported for films with a square format *(1, 3)* and circular format *(4)*, the distribution of cells on circular films appears to introduce no important bias. The method used in this study was not identical to the study of Smith *(4)*. This could explain the reason why this study revealed no important bias because of uneven distribution of cells.

It appears from results of this study that variance of counts will be lower using a wide strip than when a narrow strip is used. It would appear that a band width which would yield a working factor of approximately 10,000 would be satisfactory. Even though 450 x magnification yielded counts approximately equal to those at 1,000 x, a greater magnification than 450 x would reduce operator fatigue because of better resolution of detail. The higher magnification may permit more accurate identification of cells, especially in questionable situations.

The components of variance for bulk sub-samples and films were computed to give an indication of how these factors affect confidence limits (Table 6). It should be noted that if four films are examined using one strip on each film, the 95% confidence limits for a mean cell count of 100 are ± 11.3 cells. However, if two films are examined through two diameters

(horizontal and vertical) the confidence limits are raised to ± 15 cells. It would appear that in situations where narrower confidence limits are desired, as in research investigation, for an equivalent amount of microscope work, it may be adantageous to use four films and count one strip on each rather than two films with two strips each.

It would appear that the DMSCC has achieved the goal of offering a standardized method that permits determination of and control over precision of counting cells in milk films.

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THE CYCLAMATE STORY[1]

In spite of the effort made to clarify the events leading up to the ban on cyclamates—and the roles of the various organizations—confusion, misconception and misinformation still abound. For example, a CBS commentator, in observing the half-year anniversary of the ban, still referred to the misinformation widely disseminated earlier that the ban was based on the finding of bladder cancer in six of twelve rats fed cyclamate. Several renowned scientists, too, have made statements in the scientific and lay press based on a similar misunderstanding of the facts.

Perhaps, in once more setting the record straight, we should start by making unmistakably clear the role of FDRL, especially to point out that our involvement was solely with the sponsor of the research, Abbott Laboratories—and our reporting of findings was solely to the sponsor—and that FDRL was not in any way a party to the discussions which led to the decision by the Secretary of Health, Education and Welfare that cyclamates were no longer GRAS (generally recognized as safe under the conditions of use) and should be phased out of foods.

In a preliminary note in *Science* of February 20, 1970, the data leading up to the banning order were recapitulated. That article was signed by two scientists from Abbott Laboratories (J. M. Price, C. G. Biava), two from FDRL (B. L. Oser, E. E. Vogin), one from HEW (J. Steinfeld) and by former Commissioner H. S. Ley of the Food and Drug Administration. It recounted the earlier FDA findings that cyclamate fed for two years to rats at 1% or 5% concentration in the diet produced no effects at the lower dose and no distinct toxic effects at the higher dose. It mentioned results of research sponsored by Abbott Laboratories which showed an incidence of bladder tumors in Swiss mice which had received, by surgical implantation into their urinary bladders, pellets of 4 parts cholesterol and 1 part cyclamate. Scientists at the National Cancer Institute and the FDA agreed that this route of administration is too far removed from oral ingestion to be a suitable indicator of hazard from cylamates in foods.

Independently of such special tests, Abbott Laboratories in 1967 initiated two 2-year studies, one on cyclohexylamine sulfate at low dosage levels (conducted at another independent laboratory), and the FDRL study of a 10:1 mixture of sodium cyclamate and sodium saccharin (C/S) at daily dosage levels of 500, 1120, or 2500 mg/kg body weight, employing 80 rats on each dose. In the cyclohexylamine tests which were designed to study possible toxicity of the cyclohexylamine that may be present as an impurity in commercial cyclamates, one bladder tumor was found.

It must be emphasized that the FDRL study was designed to investigate the safety of a mixture of a

[1]Reprinted from Food and Drug Research Notes.

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ISOLATION OF SALMONELLAE AND OTHER AIR-BORNE MICROORGANISMS IN TURKEY PROCESSING PLANTS[1, 2]

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ABSTRACT

The air in seven Minnesota turkey processing plants was analyzed microbiologically for total numbers of bacteria per cubic foot, number of coliform organisms per cubic foot, and the presence of salmonellae. Plant environmental samples, that is, walls, floors, and equipment adjacent to air sampling areas, also were tested for the presence of salmonellae. Areas sampled included: live bird area (hanging, stunning, killing), scald and picking areas, evisceration line, packaging line, and further processing.

Total numbers of bacteria in the air of one plant (T-5) ranged from more than 200,000/ft^3 in the live bird area to 1/ft^3 near the evisceration line. Coliform numbers in the air of a second plant (T-1) ranged from 400/ft^3 in the live bird area to none in 100 ft^3 in the other areas of the plant. Similar results were obtained in the other plants.

Salmonellae were found in the air of the live bird area in 6 of the 7 plants and in the picking room of the other one. In each of the 7 plants salmonellae were not recovered from the air in other processing areas. The data indicate that salmonellae are frequently found in the air of areas where live birds are handled but difficult to isolate or find in other areas of the plant.

Outbreaks of food-borne disease caused by salmonellae often are traced to contaminated turkeys or turkey products. It is well known that live turkeys can harbor these microorganisms, but routes of contamination from the live bird to the dressed bird have not been definitely established.

A recent publication by Bryan et al. *(1)* suggests that *Salmonella* dissemination starts on the farm; these bacteria are brought to the plant by the incoming turkeys and transferred to equipment and turkey meat during processing. An earlier report by these same authors *(2)* indicated extensive *Salmonella* contamination of further processed turkey products. They suggested that further processed turkey products, if inadequately cooked by the consumer or if improperly handled between time of manufacture and consumption, could directly transmit salmonellae. These same products also might contaminate other foods by introducing salmonellae into food preparation areas.

While there is limited information available on the occurrence of salmonellae in turkey products and processing plant environments, there is relatively little information existent concerning the role air or air currents may play in dissemination of these organisms through a turkey processing plant. In 1964, Kotula and Kenner *(4)* reported on an investigation of air-borne microorganisms in poultry processing plants. They suggested that air may be a source of spoilage microorganisms. These authors showed variations in numbers of bacteria in various areas in the processing plant. However, no attempt was made to identify possible pathogenic organisms from this source. This project was initiated to investigate air as a possible source of potentially pathogenic and spoilage microorganisms in a turkey processing plant.

EXPERIMENTAL METHODS

Air sampling apparatus

Two types of air samplers, the Lundgren electrostatic air precipitator (LEAP) (Model 3400) and the Reyniers slit sampler[1] (Model 100 A), were used in this study. The Lundgren or LEAP sampler collects air-borne particles in a liquid menstruum whereas the Reyniers relies on impaction of the air-borne particle on a solid agar surface.

In the operation of the LEAP sampler air is passed through an electrical field which gives the air-borne particles a negative charge. Immediately below the air stream is a rotating disc which is maintained at ground potential. The particles are attracted to the disc which is covered with a thin layer of liquid. The liquid traps the particles and they are washed off the disc to a rotating dish. The liquid containing the entrapped particles is aspirated out of the dish into a sterile sample bottle. At peak capacity, the LEAP samples 40 ft^3 of air per minute.

The Reyniers slit sampler deposits air-borne particles directly on an agar surface. Air is pulled through a slit opening (slit width 0.152 mm) and particles are impacted on the agar surface 2 to 3 mm below the slit opening. A 150 x

[1]Paper No. 7213, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2]This research was supported in part by a grant from the Minnesota Turkey Research and Market Development Board.

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[1]The Lundgren electrostatic air precipitator was obtained from Environmental Research Corporation, 3760 North Dunlap Street, St. Paul, Minnesota. Reyniers slit sampler was obtained from Reyniers and Son, 3806 North Ashland Avenue, Chicago, Illinois.

20 mm culture plate containing an agar medium is rotated below the slit opening by means of a clock mechanism. After sampling, the culture plates can be incubated directly and the colonies that develop counted. The Reyniers device samples 1.5 ft³ of air per minute *(5)*.

Air sampling procedures

At the beginning of each sample period (usually 1 day) the samplers were sanitized in the following manner. The LEAP was sanitized by pumping a solution of 500 ppm sodium hypochlorite through the system for 5 min. The residual chlorine was neutralized by pumping through the system sterile 0.1% sodium thiosulfate for 5 min. The thiosulfate solution was followed by a sterile water rinse. A portion of the first collection medium through the system was collected and served as a sterility control. The LEAP was cleaned after each day's sampling.

The Reyniers sampler was sanitized before use by swabbing the interior of the sampler with 500 ppm chlorine solution.

Those two samplers were operated in close proximity to each other throughout each of the seven plants. The areas were sampled in the following order: (*a*) further process (deboning and preparation of rolled roasts, and other turkey products); (*b*) packaging (birds put into package, Cryovac bags, etc.); (*c*) evisceration (removal of viscera); (*d*) picking and scalding (feather removal); and (*e*) hanging, stunning, and killing (live bird area). This order of area sampling was followed so that the samplers were moved from areas of low particle density to areas of higher particle concentration.

The liquid sample obtained from the LEAP apparatus was collected in a sterile bottle in an ice bath and maintained cold (4 C) until analyzed. It was then divided into several portions for analysis whereas the culture dishes containing agar media which had been exposed in the Reyniers sampler were incubated directly.

Microbiological methods

To assure maximum utilization of the LEAP sample it seemed advisable to use a liquid collection medium which would not be injurious to the entrapped cells, one that could be used as a growth medium and one that could be pumped through the LEAP system over long time periods without creating problems. Several media were tried and 0.1% peptone water was selected for use as it met all the criteria given above.

The liquid samples obtained from the LEAP sampler were analyzed for number of aerobic bacteria, number of coliforms, and presence of salmonellae. Total number was determined by plating appropriate dilutions of sample with Standard Plate Count Agar (Difco). The plated sample was incubated at 32 C for 48 hr and the total number of colonies determined. This count was converted to total number per cubic foot of air by multiplying the count per milliliter of collection medium by the number of milliliters of medium collected during a time period and dividing this figure by the cubic feet of air sampled. For example:

a. LEAP sampled at rate of 40 ft³ of air per minute for 30 min
   Total cubic feet of air sampled = 30 x 40 = 1200
b. Number of colonies per milliliter of collection medium = 600
c. Total amount of liquid collected in 30 min = 60 ml
d. Calculation: Total number of bacteria per cubic foot $= \frac{60 \times 600}{1200} = \frac{36000}{1200} = 30$

The number of coliform organisms per milliliter was determined by plating appropriate dilutions of sample with Violet Red Bile Agar (VRB) (Difco). The plates were then incubated at 32 C for 24 hr. Typical coliform colonies that developed on VRB were counted and the number of coliform organisms per cubic foot was calculated in the same manner as described above.

Analyses for salmonellae were carried out in the following manner. A 10 ml portion of the sample was inoculated into 100 ml of sterile 10% reconstituted milk containing 0.002% brilliant green (Difco). The remainder of the peptone water also was used as a pre-enrichment. Both of these were incubated at 35 C for 24 hr. After this incubation period 10 ml of each pre-enrichment culture was transferred into 100 ml of tetrathionate broth (Difco) containing 0.002% brilliant green and 0.98% iodine. The enrichment cultures were incubated at 35 C for 24 hr. At the end of this time period, a generous loopful of each enrichment culture was streaked onto Xylose Lysine Brilliant Green agar (XLBG) (Difco), Bismuth Sulfite agar (BS) (Difco) and Brilliant Green Sulfadiazine agar (BGS) (Difco). The *Salmonella* analysis was completed as suggested by Galton et al. *(3)*.

A non-selective agar medium, either Standard Plate Count Agar or Trypticase Soy Agar (BBL), was used as the collection medium in the Reyniers sampler. After the samples had been taken, the culture plates containing the agar were incubated at 32 C for 48 hr. At that time, all the colonies that developed were counted, and the number per cubic foot calculated as described below. Salmonellae and coliform determinations from the Reyniers samples were carried out using a velvet transfer technique. The colonies that developed on the non-selective agar were transferred with a piece of sterile velvet attached to a wooden cylinder of appropriate size to a second culture dish containing either Eosin Methylene Blue Agar (EMB) (Difco) or XLBG. The EMB plates were incubated at 35 C for 24 hr and then checked for the presence of typical coliform colonies. On occasion, the initial and the transfer plate were overcrowded with colonies and impossible to count. When such a condition existed, total count was estimated and coliform presence was indicated by positive or negative rather than a number. The count obtained was converted to numbers of organisms per cubic foot by dividing the number of colonies counted by the total cubic feet of air sampled. For example:

a. Total numbers
   Reyniers sampler was run for 30 min at 1.5 ft³ per minute. Total cubic feet of air = 30 x 1.5 = 45 ft.³
   Number of colonies on non-selective agar after incubation = 900
   Total number of bacteria per cubic foot $= \frac{900}{45} = 20$
b. Number of coliform organisms
   Number of typical coliform colonies on EMB = 45
   Number of coliforms per cubic foot $= \frac{45}{45} = 1$

The presence of salmonellae in the Reyniers samples was ascertained by incubating the XLBG transfer plates at 35 C for 24 hr. Suspect colonies were handled in the manner described previously to verify the presence of salmonellae.

Environmental samples were taken throughout each plant and tested for the presence of *Salmonella*. The samples were taken with sterile swabs wetted with lactose broth. The cotton tips of the swabs were broken into the tubes of lactose broth and this was utilized as the pre-enrichment culture. The remainder of the *Salmonella* determination was carried out in the manner described above.

RESULTS AND DISCUSSION

The determination of the microbial content of air has been applied mainly to hospital environment, clean rooms in space technology, and less often to food processing operations. It is obvious that the microbial populations encountered in each of these situations could be quite different. Consequently, procedures that can be utilized in one situation may not necessarily be suitable for another. The first step in the development of this research program was the study of several types of air sampling devices to select a system that would provide reasonable sampling efficiency.

Microbiological contaminants occur in the air as aerosols, which are defined as solid or liquid particles suspended in the air. Particulates in an aerosol usually vary in size from less than 1μ to approximately 50μ or possibly larger. These particles may consist of single unattached organisms or may occur in the form of clumps containing a number of bacteria. The organism may adhere to a dust particle or may exist as a free floating particle surrounded by a film of dried organic or inorganic material. The objective of this study was to determine the number and type of viable bacteria in the air at various sites. Therefore, it was essential that the air sampling technique be such that the majority of the small airborne particles, 5μ or less, be collected. The two air sampling devices selected for this study met this criterion. According to the manufacturers' specifications the Lundgren Electrostatic Air Precipitator collects particles of 1μ or less at peak capacity with 80-90% efficiency. The Reyniers slit sampler collection efficiency is close to 80% with particle sizes of 5μ or less *(5)*. The use of 2 different types of air sampling apparatus (liquid entrapment, agar impaction) provided us with a collection system that provided reasonable sampling efficiency.

Seven turkey processing operations located in different areas throughout the state of Minnesota were chosen for evaluation. The data obtained in each of these plants are summarized in Table 1.

The data shown in Table 1 indicate that in each of the plants, high numbers of air-borne bacteria were associated with areas in which the live birds were hung, killed, scalded, and picked. It was in these areas that salmonellae were recovered from the air. The greatest concentration of air-borne coliform organisms also was found in these areas. The numbers of bacteria were much lower in the other areas of the plants. Apparently the flapping of the birds during hanging, the stunner, and the defeathering machines, all contributed to the development of a microbial aerosol of high concentration in the areas.

In the majority of the plants evaluated, a positive air pressure was maintained within the building; that is, the air came into the plant in the center and flowed outward. This practice is intended to prevent the flow of air from the live bird area to the processing area, to minimize the possibility of bacterial aerosols coming into contact with a dressed bird. In some of the plants studied, this air flow design was not observed. However, the data obtained from these plants showed patterns of air-borne microorganisms similar to those observed in plants which maintained positive air pressure. However, in the plants without positive air pressure distinctive separation of processing areas was evident which may have the same effect as maintaining positive air pressure.

The numbers of coliform organisms recovered varied markedly throughout the areas sampled. In some instances, none were recovered; in other areas, low numbers, 1 to 10 per 100 ft^3, were found, and in the live bird area, numbers recovered ranged from none to 400/ft^3. This wide range in numbers of coliforms, particularly in the live bird area, is difficult to explain. The inability to recover air-borne salmonellae from the live bird area may indicate that these organisms were absent from the flock being processed.

This suggestion is in agreement with data shown by Bryan et al. *(1)*. These investigators, in an extensive study of salmonellae associated with turkeys and processed carcasses, indicated that when flocks are contaminated with salmonellae on the farm, these same salmonellae can be recovered from the carcasses and the environment of the processing plant. They also were able to show that turkey flocks free of salmonellae prior to killing and dressing may become contaminated in the processing plant. It appears to us that the microbiological condition of the flock to be processed has a direct effect on the type of flora encountered in the plant during processing. The microflora associated with the dressed birds and/or further processed products may be a reflection of the types of microorganisms associated with the original flock of birds.

It is common knowledge among turkey processors that the amount of stress the bird undergoes before slaughter affects the amount of flapping during hanging and the amount of fecal material released during the operation. A hungry, thirsty bird, thus doubly stressed, will release more fecal material during the stress of hanging and this undoubtedly contributes to the microbial load of the aerosols associated with the live bird area. This may help explain some of the differences observed in numbers and types of air-borne microorganisms recovered from the live bird area.

TABLE 1. SUMMARY OF MICROBIAL ANALYSES OF AIR SUPPLIES IN 7 TURKEY PROCESSING PLANTS IN MINNESOTA

Plant	Sampling area	Salmonellae	Total number (Range per ft³)	Coliform count (Range per ft³)	Total volume air sampled (ft³)
T-1	Hanging	+[a]	13,000-150,000	1-400	17,000
	Picking	−	Over 30	Positive	5,000
	Evisceration	−	Over 30	0*-9	32,000
	Further processing	−	Over 30	10*-28	42,000
	Offal Room	−	32-36	9*-30	15,000
T-2	Hanging	+[a]	160-8,000	0*-3	14,000
	Picking	−	—	—	500
	Evisceration	−	13-19	0*	3,000
	Further processing	−	4-62	0*	5,000
T-3	Hanging	+[a]	510-7,700	4*-30	15,000
	Picking	−	Over 30	Positive	1,000
	Evisceration	−	3-120	0*	15,000
	Packing or Cryovac	−	16-82	0*-3*	15,000
	Spice mixing area	−	65	0*	2,000
T-4	Hanging	−	2,000-12,000	10-35	13,000
	Picking	+[b]	340-4,600	46*-50	17,000
	Evisceration	−	33-94	4*-2	17,000
	Packing or Cryovac	−	58-500	1*-6*	21,000
T-5	Hanging	+[a]	360-250,000	38*-180	11,000
	Picking	−	Over 56	Positive	900
	Evisceration	−	1-1,900	1*-31*	10,000
	Packing or Cryovac	−	120	0*	5,000
	Further processing	−	130	0*	4,000
T-6	Hanging	+[a]	87,000-240,000	33-130	9,000
	Picking	−	Over 56	Positive	700
	Evisceration	−	31-130	0*-2*	15,000
	Packing or Cryovac	−	6-31	0*-7*	10,000
T-7	Hanging	+[a]	12,000-37,000	54-110	16,000
	Picking	−	—	Positive	700
	Evisceration	−	1,200-1,300	1-2	6,000
	Further processing	−	3-33	0*	4,000

[a]Detected with LEAP sampler
[b]Detected with Reyniers sampler
* = per 100 ft³ air sampled

TABLE 2. SALMONELLAE SEROTYPES ISOLATED FROM TURKEY PROCESSING PLANTS

Plant No.	Source	Serotype	Number of isolates
T-1	Environmental, evisceration room	*S. bredeney*	2
T-1	Air, hanging room	*S. heidelberg*	1
T-1	Air, hanging room	*S. newport*	1
T-2	Air, hanging room	*S. godesberg*	2
T-2	Air, hanging room	*S. senftenberg*	3
T-2	Air, hanging room	Group E_2	10
T-3	Air, hanging room	Group B	4
T-3	Environmental, evisceration room	*S. typhimurium*	5
T-3	Environmental, picking room	*S. heidelberg*	1
T-3	Environmental, evisceration room	*S. anatum*	1
T-4	Environmental, picking room	*S. senftenberg*	1
T-4	Air, picking room	*S. albany*	5
T-5	Air, hanging room	*S. chester*	1
T-5	Environmental, picking and evisceration room	*S. chester*	2
T-6	Air, hanging room	Group E	2
T-7	Air, hanging room	Group E_4	2
T-7	Environmental, picking room	Group E_4	1

In each plant, during the air sampling period, at each sampling location one or two environmental samples were taken. A complete evaluation of the entire plant environment was not carried out as the interest was mainly in the air supply and the environment immediately adjacent to the air sampling area. The results are summarized in Table 2. Only those samples in which salmonellae were found are listed in the table. In addition, included in the table are the *Salmonella* serotypes isolated not only from the environment but also from the air. As can be noted from data in the table, salmonellae were more readily isolated from the environment in areas where contact with turkey viscera, feathers, and waste material was evident. In most instances, where salmonellae were isolated from the environment, contact of the sample area with one of the above had occurred.

Perusal of salmonellae serotypes listed in Table 2 shows that in only one plant (T-5) and possibly a second (T-7) were the same serotypes isolated from both the air and environment. This observation is somewhat in agreement with the data of Bryan et al. *(1)*. These investigators were able to isolate one or more different serotypes from the turkeys and contact areas during the processing operation. The original source of the different serotypes is difficult to pinpoint, but it can be assumed that these organisms originated at one time during the daily process with either the live bird, viscera, feathers, or waste material at one time associated with the live bird.

It is our contention that these data emphasize that to prevent contamination of the dressed birds and/or further processed products with air-borne microbes, it is essential that the live bird area be physically separated from the evisceration line and that this in turn be separated from the further process area. Such separation should minimize air-borne microbial contamination problems.

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THE CYCLAMATE STORY

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cyclamate salt and a saccharin salt—rather than of cyclamate alone. Although there was and still is no apriori reason for suspecting an interaction between these sweetening agents, each of which had been investigated exhaustively as separate entities in the past, the mixture which had come into common use had not been subjected to long term feeding studies in rats. When it was found during the study that rats could metabolize a small proportion of the ingested cyclamate to cyclohexylamine, the test protocol was modified to add cyclohexylamine to the diet of some of the animals in each test group. No tumors were found in the controls or in the groups on the two lower doses. At the highest dose (equivalent to 5% of the diet), a total of eight bladder tumors were observed out of the 70 rats which continued beyond one year (10 of each group having been sacrificed for histological examination at that time). FDRL's and other consultant pathologists diagnosed half or more of these tumors as carcinomas, six of the eight being visible only microscopically. Five of the tumors occurred in rats fed the C/S mixture for two years and three in rats that died after 1.5 years. All but one of the affected animals showed the capability of converting cyclamate to cyclohexylamine to some degree.

The article concluded: "There is no evidence that the use of cyclamate or saccharin has caused cancer in man, malformations in children, or any other abnormality in humans other than a rare skin hypersensitivity. However, in view of the requirements of the Delaney clause of the Food Additives Amendment, the removal of cyclamates from the classification of substances generally recognized as safe resulted in the prohibition of their use in general purpose food products."

Criticism by scientists of the ban, and of the way in which it was announced, was prompt and vocal. It was pointed out that cyclamates had been in use by humans for over twenty years without a single report of an adverse metabolic effect and without an increase in incidence of bladder carcinomas during the period; that cyclamate showed no deleterious effect when incubated with human cell cultures; that

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A COMPARISON OF HORIZONTAL VERSUS VERTICAL MIXING PROCEDURES AND PLASTIC VERSUS GLASS PETRI DISHES FOR ENUMERATING BACTERIA IN RAW MILK[1]

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Abstract

A comparison was made of a horizontal versus vertical shaking procedure for raw-milk dilutions used in the Standard Plate Count for the enumeration of milk bacteria. No significant differences ($P<0.01$) were found. Glass and plastic petri dishes were also compared in a like manner and no significant differences could be detected.

The method recommended by *Standard Methods (1)* for mixing milk dilutions consists of 25 vertical cycles of 1 ft length to be completed in a 7 sec period. This method was shown to give higher bacterial counts than two gentler inversion methods *(2)*. The vertical movements, however, are rather tiring when handling a large number of milk samples. A horizontal mixing procedure would be far easier to perform for extended time intervals. The experiments embodied in this report were set up to determine whether differences exist between vertical and horizontal mixing procedures.

A preliminary study was made by one of the laboratories cooperating in this study which indicated that glass petri dishes gave higher plate counts than plastic dishes. This was a disturbing report and indicated that a definitive comparative study of these two types of petri dishes was needed for the standard plate count since both are now in widespread use. The second part of these experiments, carried on in conjunction with the vertical and horizontal mixing study, was therefore intended to answer the question of glass versus plastic petri dish equivalence for plating raw-milk samples.

Materials and Methods

Eight laboratories participated in these studies. Six laboratories assayed six raw-milk specimens each and two laboratories assayed twelve each for a total of sixty milk samples tested. Two of the laboratories, in addition, reported the results for two separate analysts on the same milk specimens. Samples examined included those from farm bulk tanks and commingled samples from storage tanks at the manufacturing facility. The procedures used for assaying bacterial counts were those in *Standard Methods (1)* with the single exception of the horizontal mixing procedure for the dilution bottles. The plastic petri dishes used by the different investigators were purchased with no attempt made to use the product of a single manufacturer. Duplicate petri dishes were poured for each test condition. The total plate counts were usually calculated from petri dishes showing between 30 and 300 colonies; however, in all instances counts were made from the same dilution in order to avoid introducing a possible dilution variance in addition to the method variances. The statistical analysis of the results was performed in a manner similar to that of the previous report *(2)* using a 1% level of significance. Plate counts were transformed to logarithms in order to normalize the statistical distributions. Tests for reproducibility between the two mixing methods, the two types of petri dishes, and between laboratories were performed by calculating average variances for each and testing by the F ratio using the 1% level of significance.

[1]A contribution from the Subcommittee for the Examination of Milk and Milk Products, Applied Laboratory Methods Committee, International Association of Milk, Food, and Environmental Sanitarians, Inc.

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TABLE 1. EFFECT OF MIXING METHODS AND TYPE OF PETRI DISH ON PLATE COUNTS (IN THOUSANDS PER ML)

Laboratory number	Milk sample no.	Vertical mix				Horizontal mix			
		Plastic dish		Glass dish		Plastic dish		Glass dish	
1	1	14.6	16.0	16.2	15.8	16.5	16.6	16.4	16.3
	2	5.1	5.3	5.4	5.6	6.1	5.8	5.9	5.6
	3	19.8	20.0	20.4	19.4	21.0	21.3	21.4	21.8
	4	8.3	9.0	10.7	10.0	11.5	10.0	12.2	11.4
	5	20.8	21.0	21.8	22.0	19.1	19.5	19.6	19.9
	6	25.0	24.6	26.8	25.7	27.4	27.2	30.0	29.8
Average		15.8		16.6		16.8		17.5	
2	7	6.8	5.7	6.1	6.5	6.2	5.9	6.6	5.0
	8	17.5	17.3	21.0	18.6	17.9	17.5	19.3	16.6
	9	5.0	6.4	5.8	6.2	6.7	5.5	5.7	6.2
	10	14.8	13.8	13.8	16.8	12.5	18.9	16.3	19.5
	11	8.6	9.0	7.9	10.3	9.9	8.0	8.1	7.1
	12	13.9	14.9	13.3	11.7	12.5	12.6	13.8	14.3
Average		11.1		11.5		11.2		11.5	
3	13	7.2	6.6	7.1	6.6	6.4	6.0	7.0	7.0
Analyst	14	92	85	95	84	75	79	86	78
A	15	59	57	75	69	55	51	52	67
	16	48	40	43	42	46	43	42	42
	17	88	78	88	77	69	72	68	79
	18	32	35	28	34	20	24	29	27
Average		52.3		54.1		45.5		48.7	
3	13	6.8	6.9	8.7	9.1	6.6	8.6	7.1	6.9
Analyst	14	96	87	90	82	101	90	93	89
B	15	78	85	97	85	82	79	66	60
	16	44	44	44	38	41	41	42	44
	17	101	97	115	107	86	89	100	115
	18	26	24	35	38	25	25	39	35
Average		58.2		62.4		56.2		58.1	
4	19	28.2	26.5	29.0	28.1	24.9	26.3	25.9	28.8
	20	211	206	223	227	202	205	228	230
	21	47	47	48	49	43	45	49	42
	22	6.9	7.0	6.8	7.2	6.2	7.2	7.1	8.2
	23	8.7	8.1	9.7	10.4	9.5	8.5	9.2	10.2
	24	168	142	145	145	138	130	143	140
Average		75.5		77.4		70.5		76.8	
5	25	13.4	15.1	14.7	14.5	18.3	14.2	15.3	14.6
	26	16.9	19.2	19.5	18.9	20.8	20.0	17.7	18.1
	27	4.1	5.1	5.5	5.8	4.0	5.1	6.5	6.2
	28	13.8	13.0	17.2	17.1	16.8	13.1	12.7	15.1
	29	92	106	97	80	89	104	99	76
	30	60	37	62	44	47	34	78	68
5	31	12.3	12.4	11.2	12.7	11.8	10.8	12.3	12.6
	32	21.6	18.4	19.4	21.3	16.7	18.5	13.4	15.6
	33	6.6	6.4	6.3	5.1	6.4	4.6	5.9	5.8
	34	15.2	18.0	18.4	14.9	16.0	19.2	18.3	16.9
	35	9.7	10.8	9.5	8.3	10.7	13.0	8.7	8.7
	36	7.3	7.7	4.1	4.5	5.5	6.8	4.9	6.5
Average		22.6		22.2		21.9		23.2	
6	37	10.2	10.5	11.1	8.4	8.9	8.1	8.3	10.1
	38	14.7	15.0	11.7	14.6	17.2	14.3	13.6	14.1
	39	8.0	4.8	6.4	8.0	8.1	7.2	7.3	6.3
	40	3.8	3.3	4.0	3.9	4.4	3.9	4.0	3.1
	41	3.3	3.2	3.9	3.5	3.8	4.8	2.3	2.8
	42	20.7	22.2	19.8	20.1	20.9	22.6	20.9	21.5
	43	100	120	93	102	95	103	112	95
	44	5.0	4.6	4.7	6.0	4.4	5.4	5.0	4.6

	45	13.9	13.4	10.8	12.9	13.6	13.4	11.1	13.0
	46	16.8	16.4	15.7	15.5	15.8	16.8	14.1	14.4
	47	28.4	22.9	25.6	24.2	23.9	24.2	26.5	25.7
	48	8.4	8.6	8.0	9.1	9.0	8.3	6.9	8.9
Average		19.9		18.4		19.0		18.8	
7	49	296	347	327	311	334	287	345	324
	50	49	52	56	49	54	51	51	42
	51	65	67	90	74	79	75	78	86
	52	328	313	407	395	234	259	206	237
	53	66	46	63	59	52	65	60	60
	54	90	75	92	104	87	92	101	94
Average		149.5		168.9		139.1		140.3	
8	55	27.2	25.8	22.9	23.7	27.4	24.8	26.8	25.8
Analyst	56	12.3	13.5	14.1	12.1	16.1	17.2	16.1	16.9
A	57	21.8	24.9	24.2	21.6	21.7	22.1	22.2	19.4
	58	35	38	43	40	39	44	46	44
	59	35	38	30	39	34	34	34	37
	60	12.9	14.3	14.3	14.8	14.3	13.5	15.5	13.9
Average		24.9		25.0		25.7		26.5	
8	55	25.1	26.3	26.2	25.1	23.3	22.6	23.8	22.9
Analyst	56	14.6	12.7	16.3	13.8	14.9	13.8	13.7	14.4
B	57	24.7	23.2	23.6	24.6	23.0	22.5	22.6	23.0
	58	29	37	28	39	39	31	33	37
	59	29.7	27.6	28.0	29.9	27.5	28.3	27.4	26.6
	60	12.9	14.1	12.8	13.9	13.3	12.0	12.9	14.1
Average		23.1		23.4		22.6		22.6	
Over-All Average		41.3		43.4		39.1		40.5	

RESULTS AND DISCUSSION

Average plate counts

The duplicate and average plate counts for 60 samples of milk are shown in Table 1. Two analysts assayed the same milk samples in Laboratory No. 3 and also in Laboratory No. 8. These results were further summarized by combining the counts from mixing methods and type of petri dish (Table 2). The results of the averaged plate counts in this table suggested that the vertical mix gave higher plate count than the horizontal. Actually 4 of the 10 analysts (from Laboratory No. 1, 2, 5, and 8A) found higher counts with the horizontal method than with the vertical, although the overall average of the vertical method was 5.9% higher than the horizontal. Only one analyst, however, (Laboratory No. 6) found the plastic dishes to give higher counts than the glass. The glass dishes gave an average of 4.2% more colonies than the plastic.

The question of which method or variation is "superior" is subject to some individual interpretation. It would, of course, be very desirable to be able to enumerate each individual bacterial cell in a sample. This, however, is seldom practical. A method giving higher counts would generally be considered superior to one giving lower counts. An important—perhaps the most important—criterion, however is that of increased sensitivity or reproducibility of one method over another. This aspect of these analyses will be considered later in this report.

Statistical analyses

The apparent superiority of the averaged values for the vertical mixing method over the horizontal and the glass petri dishes over the plastic was analyzed further using standard analysis-of-variance procedures. The results are summarized in Table 3. The milk samples (line A) were, as expected, highly significantly different in the variations in average plate counts of bacteria. Part of this difference was accounted for as an investigator's variance (line B). The previous report *(2)* had failed to detect this source of variation at the same level of significance. Most of the variation of the milk samples was, however, caused by the samples themselves (line C) rather than by the different laboratories.

Main effects. The analysis-of-variance of treatment effects (line D) failed to show any significant differences among the four different treatments at the 1% level of significance or, for that matter, at any level of significance. The conclusion must therefore be that no differences were demonstrated between the vertical and horizontal shaking methods or between the glass and plastic petri dishes.

Interactions. The interactions resulting from these

TABLE 2. AVERAGE PLATE COUNTS BY LABORATORIES COMPARING TWO TYPES OF PETRI DISHES AND TWO MIXING METHODS (PLATE COUNTS IN THOUSANDS PER ML)

Laboratory number	Number samples assayed	Mixing method		Type of petri dish	
		Vertical	Horizontal	Plastic	Glass
1	6	16.2	17.2	16.3	17.1
2	6	11.3	11.4	11.2	11.5
3A	6	53.2	47.1	48.9	51.4
3B	6*	60.2	57.1	57.1	60.2
4	6	76.5	73.6	73.0	77.1
5	12	22.4	22.6	22.2	22.7
6	12	19.2	18.9	19.5	18.6
7	6	159.2	139.7	144.3	154.6
8A	6	24.9	26.1	25.2	25.7
8B	6*	23.2	22.6	22.8	23.0
All Laboratories	60	42.3	39.8	40.2	41.9

*Analysts 3B and 8B assayed the same six milk samples of their counterparts, 3A and 8A.

TABLE 3. ANALYSIS-OF-VARIANCE SUMMARY OF RAW-MILK BACTERIAL COUNTS[a]

Line	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	Significant ($P < 0.01$)
A	Milk Samples	59	104.8136037	1.7765018	522	Yes
B	Investigators	7	45.8576504	6.5510929	5.78	Yes
C	Samples within investigators	52	58.9559533	1.1337683	980	Yes
D	Treatments	3	0.0079833	0.0025510	0.78	No
E	Treatments X Samples Interactions	177	0.6736908	0.0038062	3.29	Yes
F	Investigators X Treatments	21	0.1431172	0.0068151	2.00	Yes[b]
G	Investigators X Plastic vs Glass	7	0.0433172	0.0061882	1.82	No
H	Investigators X Horiz. vs Vert.	7	0.0558996	0.0079856	2.35	No[c]
I	Residual	7	0.0439004	0.0062715	1.84	No
J	Treatments X Samples Within Investigators	156	0.5305736	0.0034011	2.94	Yes
K	Error (between duplicate plates)	240	0.2775849	0.0011566		
	Total	479	105.7728627			

[a]The following ratios were used for obtaining the F values: lines A/J, B/C, C/K, D/J, E/K, F/J, G/J, H/J, I/J, J/K.
[b]F ratio was on the borderline of significance at the 1% level.
[c]F ratio was, however, significant at the 5% level.

experiments are also summarized in Table 3 in lines E-J. There was a significant interaction (line E) at the 1% level of significance. It appeared that most of this interaction occurred as a result of the lack of uniform response of the various milk samples to the four treatments. This could have resulted from differences in the numbers or types of bacteria present. The glass petri dishes, for example, might have caused the agar to solidify more rapidly thus decreasing destruction of thermally-sensitive psychrophiles. There was a slight indication of investigator bias or preference for either the horizontal or vertical mixing procedure (line H) but this was only apparent at a lower level of significance (5%) than that

TABLE 4. AVERAGE VARIANCE ESTIMATES OF BACTERIAL PLATE COUNTS AMONG TREATMENTS[a]

Laboratory number	Vertical mix		Horizontal mix		Average variance estimate
	Plastic petri dish	Glass petri dish	Plastic petri dish	Glass petri dish	
1	0.0002642	0.0001710	0.0003584	0.0001258	0.002299
2	0.0016340	0.0023372	0.0040608	0.0024754	0.0026268
3A	0.0011134	0.0013131	0.0008199	0.0015932	0.0012099
3B	0.0003983	0.0009682	0.0013516	0.0007110	0.0007677
4	0.0005972	0.0001546	0.0006847	0.0010522	0.0006222
5	0.0031861	0.0023510	0.0043954	0.0016714	0.0029010
6	0.0033103	0.0023298	0.0015023	0.0022892	0.0023829
7	0.0030690	0.0012578	0.0014480	0.0011947	0.0017399
8A	0.0008364	0.0017724	0.0005120	0.006756	0.0009491
8B	0.0015438	0.0023915	0.0011225	0.0004114	0.0013673
Average variance estimate	0.0015953	0.0015137	0.0016256	0.0012200	

[a]These variance estimates (standard deviations squared) were calculated from the pooled variance estimates between duplicate plates using logarithmically transformed counts.

decided upon for the experiment.

Reproducibility among methods

The logarithmically transformed counts between duplicate plates were translated into estimates of variance (squares of standard deviations), pooled, and averaged over laboratories and methods. The final average variance estimates are given in Table 4. The reproducibility among treatments was not significantly different using the Cochran test. There also were no significant differences among the laboratories in the precision of the results obtained when an F test of the average variances was performed. All variances were well within the variance of log plate counts suggested by Donnelly et al. *(3, 4)* for reproducibility among investigators (maximum suggested variance was 0.012 in log units).

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THE CYCLAMATE STORY

(Continued from Page 399)

bladder cancer may be induced under certain conditions by the amino acid tryptophan (a constituent of most proteins) and has also been causally related to cigarette smoking; that there is serious question whether the sensitivities to cyclamate of the bladders of humans and rats are similar in the face of evidence that they are dissimilar for a number of compounds; and that selecting as the culprit one of two compounds in a mixture (the other partner being cholesterol in the implantation studies, saccharin in the feeding tests) seems arbitrary at best. Much criticism was leveled at the Delaney clause which most toxicologists, as well as HEW Secretary Finch recognize as a dogmatic law precluding any scope for the application of reasoned scientific judgment, such as consideration of dose-response relationships. Despite these criticisms and the fact that the full report of the FDRL data has not yet appeared, the use of cyclamates has been withdrawn or restricted in every country where it had been permitted.

TEXTURED VEGETABLE PROTEINS TO ALLEVIATE WORLD FOOD PROBLEMS

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ABSTRACT

There is now an increasing awareness of, and a growing concern for future nutrition requirements of affluent nations as well as developing countries. Demand for foods of familiar texture, flavor, color, appearance, and bite has resulted in the development of textured vegetable protein products. The technology of these products makes it possible to convert nutritious but unappetizing high-protein oilseed meals to familiar foods of any culture, ethinc, or geographic requirement. The resulting products provide both the organoleptic qualities and nutritional requirements which a high-protein food must contribute to the diet.

When discussing world food problems the subject may become argumentative, controversial, or even repulsive. Population explosion, food additives, pollution, and ecology are all current and controversial subjects. All relate to the food of our future.

Scientific, chemical production of food must be developed. There is no other way to meet the growing demand for nutrition. The term nutrition, not food, is used for it is possible the day will come when housewives will not be shopping for pounds, ounces, or quarts of food but for grams, milligrams, and USP units of vitamins, proteins, and other chemical nutrient needs.

PROTEIN DEFICIENCIES

Numerous articles *(2, 6, 9)* have been written about population explosion and world food problems. They all emphasize the theme that many millions of people now living and many more millions yet unborn will die from the consequences of malnutrition. A far greater number will somehow manage to survive in a constant state of physical and mental ill health. These tragedies will be the direct result of the lack of enough high quality protein in their daily diet.

Such statements may seem to be over-dramatization. They are not. They represent a single factual translation of the handwriting on the wall for a large segment of the exploding world population. For the last several decades scientists have been sounding loud clear warnings of the inevitable, disastrous consequences of uncontrolled population growth. Until recently, these storm signals have largely gone unheeded.

The obvious solution is that the length of the food chain between sunlight, oxygen, dissolved nutrients, and man's table must be shortened and that this shortening process had better begin with all possible speed. There seems hardly any need to reiterate that an appalling shortage of food already exists in the world and that this situation can only worsen under the present ground rules. Although the total caloric food deficit is bad enough, that of high quality protein is worse by several orders of magnitude. Adequate supplies of high quality dietary protein for the existing world population are either not available at this moment, or cannot be distributed, or are being wasted in wholly unbelievable amounts in those very areas of the world which can least afford to permit this to happen.

POPULATION GROWTH

Obviously, American food technology and business know-how have much to offer to attempt to solve this problem. It is equally obvious that, if it took man a million years to reach its present population level and if this number is to more than double in the next 30 years, then the shape of this curve is the exponential one which is familiar to all concerned with the problem. The United Nations has projected that the world's population will increase from its current 3.6 billion to 6.2 billion by the year 2000 *(8)*. Certainly this curve is exponential. At this moment we are well around the upward bend of the curve and approaching the 90° slope. Since an exponential curve means that the system it is measuring will, unless firm restraining forces are applied, go completely out of control and destroy itself, thus the term "population explosion" is certainly very descriptive.

While it is true that some arresting forces are already being applied to population increase, these are not generally of the controlled, humane type which we should be intelligent enough to impose by ourselves. In everywhere except the most affluent

nations, these braking forces are the direct result of the application of the laws of survival which sooner or later apply to all living things. There is no escape, for the lower forms of life, from the rigid requirement that total numbers of population must equate to the available supply of food. For these forms of life, famine, disease, and predators are constantly in action and interaction, and are maintaining the required balance. There is no escape for man either, even though up to this point he has been able to delay matters somewhat. Until now, there have always been escape routes for man from the high population density. New continents to be opened up, tilled, and harvested offered escape from famine by migration to these new lands or by importing foods from them. These escape routes are slamming shut with rather sickening thuds. There are very few nations left who have any exportable food surpluses, and one cannot help but wonder if there will be any at all by the year 2000.

There can be no escaping the conclusion that the present and future food supply situation is a very real, very urgent and very present threat to mankind's survival over a large portion of the globe. Man must face the reality that stable fertility and declining mortality cannot co-exist permanently in a finite universe such as ours. The plain, unvarnished truth is that many people now living are doomed to die prematurely, either directly or indirectly, from the lack of adequate amounts of proper foods. A far greater number, yet unborn, will suffer the same fate. If this is questioned, pause and reflect for a moment that there are 30,000 new babies every day in India alone and that India now has more children than there are people in the United States. This vast sub-continent, with a total population greater than that of the United States and Soviet Russia combined, has 80% of her people employed in agriculture and is literally living from ship to mouth on imported grains.

CAN THE PEOPLE BE FED?

It is possible, I suppose, to be callous about this situation and to conclude that, although this is a great pity, they will not have to be fed. No one, aware as we are of the things that might be done, likes to contemplate such ground rules. Quite simply, the world population is increasing faster than any present or predictable increase in total food supply. However much the laymen may like to think so, food science or engineering is not likely to find a way out of this dilemma without the intervention of the controlling action of the natural laws which will inevitably regulate our total numbers. One could go on in this vein for a long time, but it would serve little purpose. However, before going on, let's take just one look at the most freightening aspects of the effects of the current world food supply problem which is only just beginning to receive the attention which it most certainly deserves, the generation which will tip the nutritional scales toward disaster is already here. Children being weaned at this moment will not survive, and consider those in emerging nations who will, in enormous numbers, manage to be the young adults of the 1980's. The rapidly growing body represented by that of the pre-school child has a very high demand for high quality protein represented by meat, fish, eggs, milk, and animal products. The supply of these, and the essential amino acids they represent and which are required for adequate physical and mental development are in their very shortest supply in these very countries where the population density of preschool children is at its greatest.

Even though infant mortality rates in these areas range from 40-70 times higher than those in the affluent nations, huge numbers of these mal-nourished children will somehow survive but will be physically stunted and irreversibly mentally damaged. These survivors will be those who will represent a very large segment of the world population by 1985. The political and socio-economic consequences represent a terrifying aspect of future life in this world of ours. These people are and will be confined by political, ethnic, and geographic boundaries. Because of their nutritional history, they can be expected to be ready converts to communism or any other ism, however fradulent, which can be represented so as to offer even partial relief to their anguish.

TROUBLE AMONG THE AFFLUENT

It serves no purpose at all to consider this prospect as one of either academic or humanitarian interest from our current affluent vantage point. Even the most affluent nations will be in deep trouble by the year 2000. Consider for a moment that, left untouched, the population of the United States will be well over 400 million as opposed to its current 200 million, therefore an entirely new United States will have to be superimposed on the same real estate. Consider also the enormous appetite which freeways, airports, suburban sprawl, and all of the other parameters of expansion will have for arable land in this country. Let's remember also that our potable water usage has shot up from 6% of the total available amount in 1900 to 65% at this time, will approximate 85% by 1980, and that all of the available above and below ground water supply will be in full demand by

the year 2000. Lake Erie is now the world's largest open cesspool, which will take an inestimable amount of time and money to begin to correct. The Western Interstates Water Conference has warned that five of the seven great drainage areas of the Western States will be developed as far as possible within the next 15 years. We may be affluent, but we had better not be complacent.

WHAT CAN BE DONE?

What are the things which can be done, both immediately and on a longer range basis? Obviously, better fertilization, conservation measures, elimination of wastage and losses are all approaches to which the engineer can make a most worthwhile contribution. In the overall picture, however, no significant progress can or will be made in the newly independent nations until there is an adequate industrial base established which, as one well knows, is an enormously complicated task and which will take far more years to accomplish than there are available before a major crisis will have developed.

The obvious conclusion that must be drawn is that the food chain on which man depends must be shortened and made more efficient. All food has its beginning in sunlight, air, water, and dissolved nutrients. The chain of events which brings it to man's table is very lengthy and complex. The sea, bountiful as it may be, is a typical example of this process. Plankton growing at or near the surface provide food for very small fish. These, in turn, are eaten by larger fish and so on up the ladder of increasing size. Each step in the process has an efficiency no greater than 10%. The net result is that a pound of halibut is derived from 1000 lb. of original food. What solutions are on the horizon?

Let engineers and chemists get their hands on protein, no matter where it is, or in what form, and they can, at worst, hydrolyze it to amino acids or, more desirably, convert it to the most acceptable food material which is still protein and which either retains, or can be induced to regain, the familiar forms of acceptable foods. Protein powders or concentrates are the easiest to make and of course, they are pretty dull items as far as food goes.

THE OCEAN AS A PASTURE

In spite of the long food chain, the ocean is an impressive source of some of the very highest protein, and yet much of its potential remains relatively untapped. Ways must be found, and this should not be too difficult, to harvest the ocean as a controlled pasture. This would require only some minor meddling by engineers, followed by chemists converting the total fish harvest to a fish protein concentrate. The resultant bland powder is 80% or better of the highest quality protein and second only to whole egg in that respect. The balance of such a powder contains the common dietary mineral essentials as well as the important trace minerals whose dietary role is only beginning to be appreciated and which are present in such a concentrate in the correct pre-selected ratios. Here then is another valuable powder or concentrate. Currently, fish protein concentrate has not been fully approved for human consumption in this country, largely for esthetic reasons. Happily, the Bureau of Commercial Fisheries and Harvard University are actively researching processes and products to make fish protein concentrate fully approved for human consumption.

PROTEINS FROM OIL

Let's suppose we are in a country which has no coastline from which to harvest fish and not enough arable land or water for tillage and harvest. What can be done in these areas? It is still a bit "far out," but bacteria and certain yeasts can be raised on crude oil. These little rascals are a headache to refiners since they plug up refinery plumbing and are a worry to jet pilots since they will grow, if permitted to, in fuel tanks. Let's put them to work since they will, under proper conditions, grow happily on crude oil and, in doing so, chew up undesirable waxes and, in fact, partially refine the oil.

Ridiculous? Proteins from crude oil? Not at all. It has already been demonstrated that 1000 lb. of these organisms will multiply to 5000 lb. in 24 hr and, when harvested, will provide a bland powder which is 50% or more high quality protein and contains a respectable vitamin level as well. This is not a distant pipe dream. Pilot plants are turning out this material right now, and thus we have still another edible powder *(1)*.

"REAL" FOOD FROM POWDERS

People, even starving people, are funny about food, as one well knows. If it doesn't look like, taste like, or smell like familiar food, it is difficult to even give away and next to impossible to induce people to eat it on a continuing basis. This is particularly true if all you can make out of it is some gruel or similar sloppy mess. Texture, bite, mouth disappearance on mastication, or the term organoleptic qualities are enormously important in the acceptance of food.

General Mills and several other companies are well on their way to overcoming these objections and to making available analogs of familiar foods from

any or all the powders that have been mentioned. This can be done by combining such powders with a textural ingredient which can be spun, by familiar textile approaches, from the common oilseeds such as soybeans, corn, cottonseed, peanuts, sunflower, safflower, sesame, or on down the list.

These vegetable proteins in the form of solvent extracted "meals," grits," or "flours" have been around for sometime, but as has been said before, no matter how nutritious a food is, if it is unpalatable, unfamiliar, or unrewarding to eat, it will be shunned by the affluent and ignored by the needy. The way to overcome this is to convert these unappetizing powders into appetizing items which are familiar, pre-cooked, and are easy to prepare.

TEXTURED VEGETABLE PROTEINS

With this aim in mind, General Mills, and several other companies are actively researching, test marketing, and bringing these products into commercial production. Initially the approach has been to minimize the expected resistance to this new class of foods by approximating the sensory characteristics of meat, fowl, and fish. This, of course, is a purely arbitrary limitation, dictated by marketing strategy. From the technical point of view, there are almost no limitations to the reproducibility or the creation of new, never before considered categories for future introduction. In the field of food research, these developments are quite akin to the results being achieved in space science and exploration.

Briefly what is done is this *(3, 5, 8, 10)*:

(a) Defatted soy flour (52% protein) is upgraded to 97% pure soy protein by alkaline and acid extraction, each followed by centrifuging.

(b) The pure soy protein is dispersed in alkali and then precipitated at its isoelectric point in an acid bath by drawing it away, continuously, from the face of the spinnerettes, much as one would for rayon or nylon. The fiber is then washed and squeezed to remove excess water. The diameter of the filaments can be varied from 0.010 to 0.030 inch. These monofilaments have not been given any chemical hardening or other treatment and are perfectly digestible.

(c) These fibrils are combined with such standard, edible items as wheat gluten, egg albumin, vegetable or animal fats, flavors, and dyes.

(d) The mixture is then cooked, which sets up the mass.

(e) The resultant products are used as such, or refrigerated, or frozen, or canned, or if shelf stability is desired, are dried.

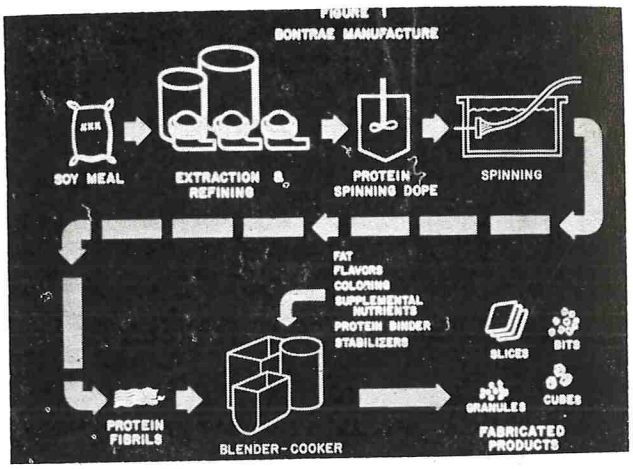

Figure 1. Process for making a number of BONTRAE vegetable protein products.

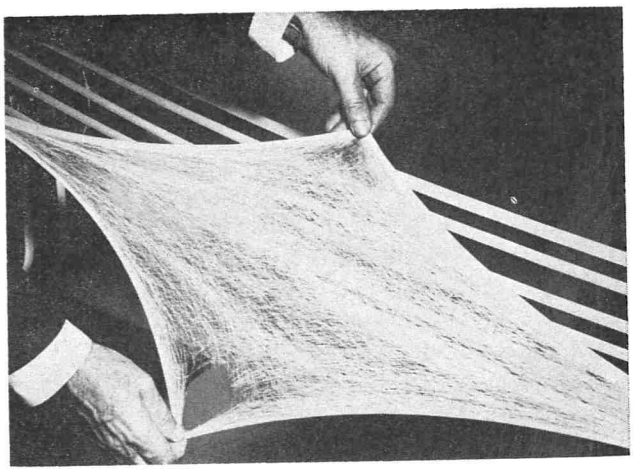

Figure 2. Expanded tow of protein fibrils.

Figure 3. Food products cooked with BONTRAE vegetable protein products.

Figure 1 represents a simplified flow diagram of the process for making a number of BONTRAE products. (BONTRAE is General Mills' brand of textured vegetable protein product.) The versa-

tility of this process is achieved just prior to the cooking stage. At this point conventional or unconventional supplements can be added. The fiber is intimately blended with fat, flavors, coloring, supplemental nutrients, and stabilizers and bound together with a heat coaguable protein. After fabrication, individual products can be sliced, ground, or diced. In this state they are analogous to cooked meat and, with some exceptions, are handled in the same fashion. Figure 2 shows an expanded view of a tow of fiber which contains 16,000 individual fibers.

Figure 3 shows typical dishes prepared from BONTRAE. General Mills' dehydrated bacon analog is in national distribution in the supermarkets. Frozen analogs of beef, ham, and chicken have been in a test market to hotels, restaurants, and institutions for over 18 months. General Mills' commercial production facility is being built in Cedar Rapids and will be in full production by summer of 1970.

Most of the work to date has utilized soy protein because of its economy and nutritional quality. But other vegetable animal proteins can be spun. Egg albumin is the most widely used binding agent. Fat content can be varied from essentially 0 to 50%. Low-melting or higher-melting fats of either animal or vegetable origin can be used. Additional proteins, vitamins and/or minerals may be incorporated, depending on the nutritional properties desired. Flavor and color can be provided by natural or artificial systems or combinations of both. Being a formulated and manufactured product, it is possible to tailor-make products to meet any dietary, ethnic, religious, or geographic requirements.

The nutritional qualities of these products have been adequately documented. The protein efficiency ratio has been found to compare very favorably with milk protein. General Mills had done extensive nutritional testing of these products not only in animals but human patients as well. Human feeding experiments were conducted at the University of Iowa *(7)*. In this study, complete medical histories were taken and the conclusions were that human patients do very well when BONTRAE was used as the major source of protein. Studies conducted in Guatamala *(4)* using both animals and children also demonstrated the nutritional excellence of these products.

These new products are in no sense a threat to the meat industry. These new foods compliment meat, extend and improve total carcass use. For instance, carcass portions which perform poorly in canning operations can be made to perform well by proper combination with soy fibers.

In summary, we feel that, with predictable progress in simplifying the techniques and equipment needed for their production, textured protein foods will provide a nutritious source of protein for developing countries and to supplement, not supplant, traditional animal meats in the domestic market place.

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THE MANPOWER PREDICAMENT IN ENVIRONMENTAL HEALTH[1]

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Few people would dispute that the quality of the environment is deteriorating and that corrective action requires an effective system of communications among professionals and laymen; a sound management of our available resources and strong leadership at all levels of community interest and concern.

Traditionally, our way of dealing with environmental hazards to man's health and welfare has been one of reaction to crisis. When environmental hazards became apparent, elements of our society reacted to eliminate or control such hazards or merely to live with them. This pattern of environmental protection has fallen short of its purpose, and may well be the gut issue that can unify or polarize this nation in the 1970's.

A fundamental new approach is needed: one based on the idea that man's health and welfare ought to be regarded as nature's last, not the first, stress signal of an environmental health hazard.

This approach to environmental health must be based on a new view of the relationship of man's health to his environment—namely, that human illness can be a symptom of environmental stress and that environmental health efforts must treat or prevent the disease itself, not the symptoms.

Obviously, we cannot pursue this approach in a vacuum and our capabilities to deal with environmental problems effectively and realistically depend upon a broad spectrum of resources and what we do in other areas where we face comparable crises and challenges.

In the field of environmental health, as in any human endeavor, the success in achieving stated goals is closely related to the quality of manpower brought to bear therein. The effectiveness of national, state, and local programs in environmental management depends, in no small measure, on the availability of competent personnel in sufficient numbers to meet the task we face today. So it is in this general setting that I wish to sketch in fairly broad strokes the predicament of environmental health manpower.

[1]Presented at the 20th Annual Meeting of the Pennsylvania Health Council, Philadelphia, Pennsylvania, March 9, 1970.

MANPOWER SUPPLY IS CRITICAL

Although the problem of manpower has been developing for a number of years, it is only recently that these issues have begun to take definite directions. The current supply of environmental control manpower both in engineering and the sciences, is at a critical stage. Since 1960, study after study and report after report, tell the same story of continuing vacancies in the areas of applied research and development, teaching, and in the field application of knowledge for the prevention and control of environmental hazards to health. A recent survey by the Public Health Service recognized approximately 38,000 persons employed in environmental control, an estimate based on 9,000 engineers, 15,000 sanitary scientists and supporting technicians, and approximately 12,000 environmental program specialists.

Water pollution programs

Water pollution abatement programs are in serious trouble not necessarily because of inadequate funds and technology, but deficiencies in manpower. In 1967 about 3,600 scientists and related professionally trained people were employed in water pollution control programs. Currently there is an urgent need to increase that number by more than 150% without any consideration of replacement. In Boston, operation of the Deer Island waste water treatment plant, after construction, was delayed for several months merely because adequately trained operators were unavailable. Today, St. Louis needs approximately 110 operators at its new metropolitan treatment plant which will become fully operational late this year. At the last count there were approximately 20,000 operators of waste water treatment plants; within the next two years 35,000 will be needed to help clean up our nation's streams.

In the field of sanitary engineering there is currently an annual need for 116 additional professionals to promote and protect public health through design, operation and management of facilities that control and improve man's environment.

The manpower situation in water resources is further illustrated by the recent experience of the Wa-

ter Resources Council (WRC), a federal interdepartmental agency which makes grants to states to assist in developing comprehensive water plans. In fiscal 1968 WRC disbursed 2.2 billion dollars to 49 states. Indiana alone did not participate. That State reported to the Council it did not want money because it had so many unfilled positions already that it would be pointless to take more money to create more jobs it cannot fill. Similarly, many other states, Puerto Rico, and the Virgin Islands are experiencing recruitment difficulties in water pollution control programs.

Air pollution programs

In the field of air pollution, the situation is not much better. In 1961 state agencies employed approximately 148 full-time and 30 part-time personnel. Today, several thousand vacancies exist because of shortage of qualified personnel. This shortage will certainly get worse. Already 1900 new positions have been created by abatement programs now being supported by the National Air Pollution Control Administration.

The manpower needs of industrial plants and engineering firms for air pollution control personnel push the situation beyond the serious to the critical stage.

Solid waste management

The rising national concern about solid waste adds another dimension to the environmental health manpower problem. Similar to other environmental areas, solid waste is plagued by a lack of adequately trained personnel to help develop new concepts in solid waste technology and management. The need for more refined solid waste technology and qualified personnel is brought into sharper focus when it is realized that 75% of all solid waste ends up in open dumps with the associated health hazards: flies, rats, and other vermin.

Local health services

In traditional local environmental health services as provided by local departments of public health, the level of qualified manpower is not, and never has been, commensurate with new responsibilities resulting from advances in technology and population growth. The approximately 17,000 public health sanitarians and sanitarian technicians can hardly be expected to deal effectively with problems of institutional hygiene, milk and food protection, housing hygiene, accidental injury control, land-use planning, and general community sanitation.

In addition, the expanding role of hospitals and related medical care institutions has generated the need for specialists in the field of institutional environmental control, with major concern for the health and safety of both patients and staff. The control of the physical environment in medical care facilities requires a specialist with more than the usual training in basic community sanitation. This responsibility calls for training in environmental microbiology, radiological health, epidemiology, disinfection and sterilization techniques, as well as general aspects of hospital administration. A cursory review of the "employment service section" of monthly health-oriented journals would indicate that such persons are in extremely short supply.

Any official health agency operating in today's industrial age that intends to give equal service to all citizens who support it should in theory have an occupational health and industrial hygiene program. Yet the most recent estimates available indicate that less than 3,000 industrial hygienists are employed in the United States. Most of them work in an industrial setting, in transportation or utility companies. The 50 states, Puerto Rico, and the District of Columbia employ less than 1,800 occupational safety inspectors. New York, California, and Pennsylvania account for more than 700, whereas Massachusetts, Mississippi, Nevada, New Hampshire, New Mexico, and North Dakota have an even smaller number of personnel with responsibility for on-the-job safety.

Ecology

Moving from the narrow concern of environmental health services adminstration to the broader concept of the total environment, I believe that many of the urgent problems facing and threatening mankind can be defined as an ecological crisis—a breakdown in the environmental system which governs the quality of life. Accordingly we must begin to recognize the promise that ecological considerations offers both for the prediction of and solution to environmental problems. Despite these promises however, the science of ecology also suffers, as do other environmental quality issues, from lack of manpower.

LaMont C. Cole, the distinguished Cornell ecologist, recently expressed concern that not enough scientists are being trained to implement the ecological programs that will be recognized as necessary in the near future. According to the National Register, only 1,300 biologists are identified as ecologists and less than 800 are being graduated each year from colleges and universities.

WHY THE PREDICAMENT?

At this point, one may logically ask: are there identifiable reasons for the manpower predicament of environmental health? There are some central issues that seem to be clearly identifiable.

Inadequate opportunity

In less affluent state and local areas, the quality of the program is frequently not a sufficiently challenging opportunity for professional career development. Potential candidates for employment soon lose interest in a system which limits opportunity for promotion to the top. The opportunity to advance, to conduct research, to participate in shaping of programs, and to tackle difficult problems is a necessary component of career development and job satisfaction. Undoubtedly, inadequate salaries make it difficult, if not impossible, to compete with private industry or services. For example, a person suitable for training in industrial hygiene should have a B.S. degree and, preferably, an M.S. degree in chemistry, engineering, physics, or biological science. The prevailing salary outside governmental agencies for such personnel is so much higher than that established in most health departments, that it is nearly impossible to hire well-trained personnel or to retain inductees after they have gained experience in the industrial hygiene field. It is a tragedy that one of the most serious obstacles to the recruitment and retention of environmental health personnel lies in our civil service system, originally designed to assure leadership of high quality based on merit rather than political affiliation. *These systems not infrequently have, with some justification, been accused of perpetuating mediocrity.* Rigid seniority requirements for promotion have tended to discourage those with greatest leadership potential and they have sought other outlets for their superior ability, leaving the less talented and therefore less sought after, in positions of responsibility. One occasionally hears a long-time civil servant explain his rise to a high position by the comment "I never bothered anybody, or I never rocked the boat, and nobody bothered me." Promotion was inevitable, given enough time.

Fragmentation of health services

Probably one of the most important factors has been the tendency to fragment health services through creation of competing agencies resulting in a situation in which strong state or local leadership was inhibited. One need only look at the national level for examples of fragmentation and duplication which have resulted in underutilization of environmental health manpower. No less than 13 congressional committees have "a piece of the environmental health action." In addition, there are 90 separate federal environmental programs plus 26 quasi-governmental bodies and 14 interagency committees at work on aspects of the environment. Such an arrangement creates waste and inefficient use of manpower in the form of red tape, duplication, and conflicting goals. Moreover, the federal government through its grant program, has created and financed "islands of excellance" in universities and industry in those very scientific and engineering aspects of health that heretofore had been the responsibility of the Public Health Service (PHS). Thus, expertise outside the Public Health Service has developed and now competes with the PHS as well as state and local health agencies for trained personnel who are in short supply.

The Public Health Service

Allow me to comment on another devolpment at the national level which impinges on the total environmental manpower problem. From its inception in 1789, the Public Health Service has provided leadership for meeting health problems in this country in areas or situations where there were otherwise inadequate or absent resources. However, since 1960, major reorganizational changes have taken place which have altered the status, role, and authority of the Surgeon General and the Commissioned Corps of career health professionals. These changes have created confusion and uncertainty as to the leadership role or responsibility of PHS for national health affairs that it has had in the past. As a result, a considerable number of experienced environmental health specialists of the professional Commissioned Corps have left and are leaving the Public Health Service. The remainder are somewhat disturbed and uncertain as to their own future as well as that of the Commissioned Corps. The effects of these developments on the total environmental manpower pool at all levels of government are fairly obvious.

If state and local health agencies are to serve on the firing lines for a vigorous program of environmental health, much of their immediate support must come from the national level where trained specialists must be available to provide assistance and to identify special problems. Indeed this technical assistance is very important to state and local environmental health administrators who continuously lack trained personnel and an adequate budget.

Racial discrimination

No paper, no discussion, no argument on the question of health manpower shortage can proceed very far without considering the impact of racial discrimination on the present predicament of environmental health manpower. The "quota system" is still used, sometimes in subtle form, to exclude minority groups from many of the health training institutions. Nonrelevant admission qualifications and credential barriers coupled with the bleak prospects for advancement, have served to limit the number and quality of minority group members who seek preparation and

careers in environmental health science and engineering.

Terence Carroll, Director of the National Institutes on Rehabilitation and Health Services, recently corroborated this view in a published letter to the editor of the *American Journal of Public Health*. His conclusions are worth repeating here:

> "Related to the matter of segregated health facilities was the failure of the (U.S.) Public Health Service over the years to stress the need for an expansion of training programs for health personnel and the admission of students to these programs without regard to race or religion."
>
> "The quota system which was used as an admission policy by nearly every medical school for decades, and the outright exclusion of blacks from many of the institutions that provide training in the health disciplines, has contributed substantially to the present scarcity of health manpower."

There can be no question that minority groups have long been under-represented in the "providers" segment of community health services, and this imbalance also has had a severe impact on the provision for total environmental protection as well as personal health services. All too many non-minority-group "providers" of health services are not sufficiently attuned to the needs of minority groups. The narrow concepts of these health workers and their insufficient dedication to those "across-the-tracks" have helped sustain the black-white morbidity and mortality dispartiy, the high level of rat infestation in the inner city, the substandard housing which still plagues the urban environment, and the low quality of foods offered for sale to the minority-group consumer in the urban ghetto.

It is encouraging to note that the American Public Health Association recognized the gravity of the situation and adopted a resolution last year at its Annual Meeting in Philadelphia, calling for "Equal Rights in Health Manpower." Toward this end, the association will join a national effort to prepare a health manpower policy for equal rights. I sincerely hope that these guidelines will not be just another publication in the archives of the Association.

Discrimination based on sex

Barriers to recruitment based on sex also have had their effects on the manpower problem. Women constitute an important resource for skilled environmental health manpower. Although the majority of health workers are women, they tend to be employed in lower level jobs and at a lower salary than men. There seems to be a prevailing view that there is something unfeminine about environmental health practice. Perhaps this has been generated by the aspiration and conviction of the engineer-health official that he should be the natural leader of the environmental health team. I am sure that in some regards this is very sound.

As I see the field of environmental health developing there are many areas where others who are neither male nor engineer are likely to be more competent. A basic unresolved issue is how to assure at the helm, the best qualified professional, regardless of race or sex.

Sex, racial, and ethnic heritage have no place in our society as barriers to effective recruitment of health manpower and time is slowly running out for those who would perpetuate such a system.

Imbalance in priorities

Another dimension of the real issue centers on the question of our enormous, indeed outrageous, imbalance in our national effort and in our national priorities.

Last year we spent approximately 8 billion dollars on research and development for military weapons and on the space program. Interestingly enough, some of this so called defense research had no forseeable military application. What did we spend in applied research in air pollution, waste water treatment, solid waste disposal, urban development, traffic and noise? I suppose approximately 100 to 115 million dollars.

Is it any wonder that with this kind of an imbalance, with this kind of distortion of national effort that the scientific and engineering manpower is marshalled where the money is. Thus, in recent years we have seen an increasing number of doctoral degrees awarded to students who had their sights on top research assignments in the aerospace or defense related research programs. To pursue that direction was not only human nature, it was the most natural thing to do.

Failure to redefine goals in health professions

The health profession itself also has failed to redefine its goals and to shift the resources required to meet these goals. This unquestionably implies an unsatisfactory articulation with education and training of manpower. It seems highly questionable that because physicians were asked to head up many fields of public health when communicable diseases had a priority role, that this pattern should hold when such problems as urban development, housing hygiene and safety, noise, air and water pollution, solid waste management, and other developments which are far removed from the medical curriculum, are high on the list. Our failure to rethink staffing requirements pursuant to a shifting of goals has unfortunately helped to perpetuate the present health manpower

stringency. Another contributing factor is the lack of coordination horizontally and vertically in the field of environmental health. It makes little or no sense for the environmental health specialist not to be associated with other professionals engaged in seeking solutions to current man-environment problems. Individually, each is likely to have very little influence, as we have seen in the past. Collectively, there is the strong possibility that they might be able to effect positive changes. We environmentalists need allies and must make formal alliances with urban planners, housing experts, lawmakers, conservationists, consumer groups, and related disciplines. Dr. Harlan Cleveland put it this way:

> "——nowadays, significant decisions are hardly ever made by individuals but instead by a complicated process of brokerage and bargaining, of committee work and consensus. And so the expert at whatever level has the obligation not only to be right, but also to be an effective salesman and organizers who can mobilize the work of others around his own perceptions."

We environmentalists need to be able to mobilize the work of others around our own perceptions in an effort to complement existing manpower. In some activities related to environmental health, we will be in charge; in others we will be a member of a leadership team—sometimes a dominant member, sometime subordinate.

IN CONCLUSION

In conclusion, you have heard many persons attempt to offer a panacea for the kind of problem which I have discussed in this paper. My experience in academic teaching and research and more recently as administrator of an urban environmental health program would indicate that there is no single solution which can be applied across the board.

I do not believe that the answer lies solely, or even primarily, in opening up a large number of new positions with new titles and subtitles related to new technics and new functions that emanate from new technologies. Rather I suspect that we face a more serious challenge of reassessing our goals, re-evaluating our resources, and realigning our methods in a broad systems approach.

Unquestionably, environmental health is an exciting field and full of unprecedented challenges. As such, it should be able to attract a reasonable proportion of able people. We can only achieve this goal through appropriate concern for and action on those factors which continue to frustrate our best effort to resolve the manpower predicament in environmental health.

HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON AUGUST 20, 1970

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to C. A. Abele, Secretary-Treasurer, 2617 Hartzell St., Evanston, Ill. 60201."

0102 Storage Tanks for Milk and Milk Products as Amended

116 Jacob Brenner Company, Inc. (10/ 8/59)
450 Arlington, Fond du Lac, Wisconsin 54935

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St., Little Falls, N. Y. 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets, Chester, Pennsylvania 19013

1 Chicago Stainless Equipment Corp. (5/ 1/56)
5001 No. Elston Avenue, Chicago, Illinois 60630

2 CP Division, St. Regis (5/ 1/56)
1243 W. Washington Blvd., Chicago, Illinois 60607

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

76 Damrow Company (10/31/57)
196 Western Avenue, Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street, So., Peterborough, Ont., Canada

207 The DeLaval Separator Co. (7/23/69)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

109 Girton Manufacturing Company (9/30/58)
Millville, Pennsylvania 17846

21 The J. A. Gosselin Co., Ltd. (9/20/56)
P. O. Box 280, Drummondville, Quebec, Canada

114 C. E. Howard Corporation (9/21/59)
9001 Rayo Avenue, South Gate, California 90280

127 Paul Mueller Company (6/29/60)
1616 W. Phelps Street, Springfield, Missouri 65801

197 Paul Mueller (Canada), Ltd. (9/ 9/67)
84 Wellington St., South, St. Marys, Ont.

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

0204 Pumps for Milk and Milk Products Revised, as Amended

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wis. 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd., Oak Brook, Ill. 60621

29R Cherry-Burrell Corporation (10/ 3/56)
105 W. Adams St., Chicago, Ill. 60603

63R CP Division, St. Regis (4/29/57)
1243 W. Washington Blvd., Chicago, Illinois 60607

205R Dairy Equipment Co. (5/22/69)
1919 So. Stoughton Road, Madison, Wisc. 53716

180R The DeLaval Separator Co. (5/ 5/66)
Duchess Turnpike, Poughkeepsie, N. Y. 12602

65R G & H Products Corporation (5/22/57)
5718 52nd Street, Kenosha, Wisconsin 53140

145R ITT Jabco, Incorporated (11/20/63)
1485 Dale Way, Costa Mesa, Calif. 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140

148R Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Ave., Springfield, Ohio 45501

163R Sta-Rite Products, Inc. (5/ 5/65)
343 Wright Street, Delavan, Wisconsin 53115

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 53rd Street, Kenosha, Wisconsin 53140

175R Universal Milking Machine Div., (10/26/65)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007

52R Viking Pump Div.—
Houdaille Industries, Inc. (12/31/56)
406 State Street, Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

0402 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

87 Cherry-Burrell Corporation (12/20/57)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

37 CP Division, St. Regis (10/19/56)
1243 W. Washington Blvd., Chicago, Illinois 60607

75 Manton-Gaulin Mfg. Co., Inc. (9/26/57)
44 Garden Street, Everett, Massachusetts 02149

0506 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

131 Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road, Almont, Michigan 48003

98 Beseler Steel Products, Inc. (3/24/58)
417 East 29th, Marshfield, Wisconsin 54449

70 Jacob Brenner Company (8/ 5/57)
450 Arlington, Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
600 Sixth Ave., S. E., Minneapolis, Minn. 55114

118 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

66 Dairy Equipment Company (5/29/57)
1818 So. Stoughton Road, Madison, Wisconsin 53716

123 DeLaval Company, Ltd. (12/31/59)
113 Park Street, South Peterborough, Ont., Canada

190 Eastern Industries, Limited (11/18/66)
830 Blvd. Lemire, Drummondville, Quebec, Canada

121 The J. A. Gosselin Co., Ltd. (12/ 9/59)
P. O. Box 280, Drummondville, Quebec, Canada

45 The Heil Company (10/26/56)
3000 W. Montana Street, Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
West Hill, Ontario, Canada

80 Paul Mueller (Canada), Ltd. (11/24/57)
84 Wellington Street, So., St. Marys, Ont., Canada

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minn. 56340

144 Portersville Equipment Company (5/16/63)
Portersville, Pennsylvania 16051

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street, Arthur, Illinois 61911

47 Trailmobile Div. of Pullman, Inc. (11 2/56/
16th & Howell Streets, North Kansas City, Mo. 64116
189 A. & L. Tougas, Ltée (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada
25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

0808 Fittings Used on Milk and Milk Products Equipment, and Used on Sanitary Lines Conducting Milk and Milk Products, Revised

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186
138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada
82R Cherry-Burrell Corporation (12/11/57)
105 W. Adams St., Chicago, Ill. 60603
124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South, Peterborough, Ont., Canada
184R The DeLaval Separator Co. (8/ 9/66)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
67R G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
199R Grayco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413
203R Grinnell Company (11/27/68)
260 W. Exchange St., Providence, R. I. 02901
204R Hills McCanna Company (2/10/69)
400 Maple Ave., Carpentersville, Ill. 60110
34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140
200R Paul Mueller Co. (3/ 5/68)
1616 Phelps St., Springfield, Mo. 65601
149R Q Controls (5/18/64)
Occidental, California 95465
89R Sta-Rite Industries, Inc. (12/23/68)
343 Wright Street, Delavan, Wis. 53115
73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140
191R Tri-Canada Fittings & Equipment, Ltd. (11/23/66)
21 Newbridge Road, Toronto 18, Ontario
151R Tubular Components, Inc. (11/18/64)
Butternut Drive, East Syracuse, New York 13057
215R Universal Milking Machine Div., (7/31/70)
National Cooperatives, Inc.,
First Avenue at College, Albert Lea, Minn. 56007
86R Waukesha Specialty Company (12/20/57)
Walworth, Wisconsin 53184

0902 Thermometer Fittings and Connections Used on Milk and Milk Products Equipment and Supplement 1, As Amended

32 Taylor Instrument Companies (10/ 4/56)
95 Ames Street, Rochester, New York 14611
206 The Foxboro Company (8/11/69)
Neponset Ave., Foxboro, Mass. 02035

1002 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

1102 Plate-Type Heat Exchangers for Milk and Milk Products, As Amended

20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207
30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404
14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013
38 CP Division, St. Regis (10/19/56)
1243 W. Washington Blvd., Chicago, Illinois 60607
120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South, Peterborough, Ont., Can.
17 The DeLaval Separator Company (8/30/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

1202 Internal Return Tubular Heat Exchangers, for Milk and Milk Products, As Amended

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013
96 C. E. Rogers Company (3/31/64)
8731 Witt Street, Detroit, Michigan 48209
152 The DeLaval Separator Co. (11/18/69)
350 Duchess Turnpike, Poughkeepsie, N. Y. 12602

1303 Farm Milk Cooling and Holding Tanks—Revised, As Amended

11R CP Division, St. Regis (7/25/56)
1243 W. Washington Street, Chicago, Illinois 60607
119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park, St. Cloud, Minn. 56301
4R Dairy Equipment Company (6/15/56)
1919 S. Stoughton Road, Madison, Wisconsin 53716
92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada
49R The DeLaval Separator Company (12/ 5/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846
95R Globe Fabricators, Inc. (3/14/58)
7744 Madison Street, Paramont, California 90723
179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
635 Laurel St., Preston, Ont., Canada
12R Paul Mueller Company (7/31/56)
1616 W. Phelps Street, Springfield, Missouri 65801
58R Schweitzer's Metal Fabricators, Inc. (2/25/57)
806 No. Todd Avenue, Azusa, California 91702
134R Universal Milking Machine Division (5/19/61)
National Co-operatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007
42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126
18R Whirlpool Corporation, St. Paul Division (9/20/56)
850 Arcade Street, St. Paul, Minnesota 55106
55R John Wood Company (1/23/57)
Superior Metalware Division
509 Front Avenue, St. Paul, Minnesota 55117
170R The W. C. Wood Co., Ltd. (8/ 9/65)
5 Arthur Street, South, Guelph, Ont., Canada
16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

1400 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers, As Amended

122 Cherry-Burrell Corporation (12/11/59)
105 W. Adams St., Chicago, Ill. 60603

69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140
78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

1603 Evaporators and Vacuum Pans for Milk and Milk Products, Revised

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207
111R Blaw-Knox Company (2/12/59)
Dairy Equipment Division
750 E. Perry, Buffalo, N. Y. 14210
110R Arthur Harris & Company (11/10/58)
210-218 North Aberdeen Street, Chicago, Illinois 60607
164R Mora Industries, Inc. (4/25/65)
112 South Park Street, Mora, Minnesota 55051
107R C. E. Rogers Company (8/ 1/58)
8731 Witt Street, Detroit, Michigan 48209
186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48008

1702 Fillers and Sealers of Single Service Containers, For Milk and Milk Products, As Amended

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404
139 Exact Weight Sale Company (4/15/68)
944 West Fifth Ave., Columbus, O. 43212
137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232
140 General Films, Inc. (4/23/63)
Covington, Ohio 55318
142 Polygal Company (4/15/63)
Div. of Inland Container Corp.
P. O. Box 68074, Indianapolis, Indiana 46268
210 Twinpak, Ltd. (2/ 4/70)
270 St. Joseph Blvd., Lachine, Quebec
211 Twinpak, Inc. (2/ 4/70)
1133 Avenue of the Americas, New York, N. Y. 10010

1901 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

141 CP Division, St. Regis (4/15/63)
1243 W. Washington Blvd., Chicago, Illinois 60607
146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

2201 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls, N. Y. 13365
154 CP Division, St. Regis (2/10/65)
1243 W. Washington Blvd., Chicago, Illinois 60607
160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
181 Damrow Company (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935
156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280
155 Paul Mueller Co. (2/10/65)
1616 W. Phelps Street, Springfield, Missouri 65801
195 Paul Mueller (Canada), Ltd. (7/ /67
84 Wellington St., So., St. Mary's, Ont., Canada
165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

2300 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109
209 Doughboy Industries, Inc. (7/23/69)
Machine Division
869 So. Main Ave., New Richmond, Wisc. 54017
193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

2400 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365
158 CP Division, St. Regis (3/24/65)
1243 W. Washington Blvd., Chicago, Illinois 60607
187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
208 The DeLaval Separator Co., (7/23/69)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846
166 Paul Mueller Co. (4/26/65)
1616 W. Phelps Street, Springfield, Missouri 65802
198 Paul Mueller (Canada), Ltd. (9/ 9/67)
84 Wellington St., So., St. Marys, Ont., Canada

2500 Non-Coil Type Batch Processors for Milk and Milk Products

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill st., Little Falls, N. Y. 13365
159 CP Division, St. Regis (3/24/65)
1243 W. Washington Blvd., Chicago, Illinois 60607
188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
167 Paul Mueller Co. (4/26/65)
1616 W. Phelps Street, Springfield, Missouri 64801
196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada
202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

2600 Sifters for Dry Milk and Dry Milk Products

171 Entoleter, Inc. (9/ 1/65)
Subsidiary of American Mfg. Co.
1187 Dixwell Avenue, Hamden, Connecticut 06514
173 Food & Chemical Equipment Div., (9/20/65)
Blaw-Knox Company
1325 S. Cicero Avenue, Chicago, Illinois 60650
185 The Orville-Simpson Co. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223
176 Sprout, Waldron & Co., Inc. (1/ 4/66)
Munsy, Pennsylvania 17756
172 SWECO, Inc. (9/ 1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

AMENDMENT TO 3-A SANITARY STANDARDS FOR STAINLESS STEEL AUTOMOTIVE MILK TRANSPORTATION TANKS FOR BULK DELIVERY AND/OR FARM PICK-UP SERVICE

Serial #0510

Formulated by

International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The 3-A "Sanitary Standards for Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-Up Service, Amended April 28, 1954", Serial #0501, are hereby further amended in the sections indicated below:

The following sentence is added to the DEFINITION:

Both may have more than one tank or compartment.

Substitute the following for subsection D.1:

1. *Outlet*: A milk outlet is defined as the opening in the inside lining of a tank or compartment and the outlet passage for milk to the exterior of the tank or compartment. The outlet passage starts at the opening in the inside lining and terminates at the connection for the outlet valve. Each tank or compartment shall have a separate outlet passage. The outlet shall be of all welded construction (except that a rolled-on flange may be used on the terminal end) and shall have an inside diameter at least as large as that of a 2-inch 3-A Sanitary Fitting. The outlet(s) shall provide complete drainage of the tank(s) or compartment(s). In multi-compartment or multi-tank units, the top of the outlet passage(s) of the front compartment(s) or the front tank(s) shall be as low as the low point of the inside lining at the outlet at a point in the outlet passage. The horizontal distance from the opening in the inside lining to this point shall not be more than four times the diameter of the outlet passage. The outlet passage downstream of this point shall pitch towards the connection for the outlet valve. The terminal end shall have a welded or rolled-on flanged connection for the outlet valve. The terminal end of the outlet passage shall not extend more than 6 inches beyond the inside lining of the tank or compartment(s). The outlet passage may be increased in length provided that:
   (a) The outlet passage is straight or is straight downstream of the elbow(s) or bend(s) used either to change the direction of product flow from a bottom outlet or to comply with the requirement in the preceding paragraph that the top of the outlet passage shall be as low as the low point in the inside lining at a specified point.
   (b) The outlet and outlet passage may be adequately cleaned manually or the tank or compartment with the increased outlet passage is provided with a fixed spray device(s) so that the outlet and outlet passage may be mechanically cleaned.
   (c) The outlet passage is insulated sufficiently that the temperature rise of the water in the outlet passage does not exceed the allowable average temperature rise of the tank full of water (2 F) specified in subparagraph A.4.
   (d) The outlet passage is protected against damage (denting) and is braced and sloped.

This amendment shall become effective November 24, 1970.

AMENDMENT TO 3-A SANITARY STANDARDS FOR FITTINGS USED ON MILK AND MILK PRODUCTS EQUIPMENT AND USED ON SANITARY LINES CONDUCTING MILK AND MILK PRODUCTS

Serial #0810
Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809", is amended as follows:

Substitute the following for F. and F.1:

F. *DRAWINGS OF 3-A FITTINGS*

F.1 Drawings for the following fittings are included in this Standard.

Delete the statements in Section F. that follow the fitting list.

Delete the following from the note on 3-A Drawings No. 3A-100-24 (page 21) and No. 3A-100-25 (page 22).

"THE DESIGN USED SHALL CONFORM WITH SANITARY REQUIREMENTS SET FORTH IN SUPPLEMENT 5 OF THE '3-A SANITARY STANDARDS FOR FITTINGS USED ON MILK AND MILK PRODUCTS EQUIPMENT, AND USED ON SANITARY LINES CONDUCTING MILK AND MILK PRODUCTS.' "

This amendment is effective November 24, 1970.

AMENDMENT TO 3-A SANITARY STANDARDS FOR MULTIPLE-USE PLASTIC MATERIALS USED AS PRODUCT CONTACT SURFACES FOR DAIRY EQUIPMENT

Serial #2004
Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", are hereby amended as indicated in the following:

Section I. Standards for Acceptability, Sub-paragraph (2):

Add the following material to the list of Generic Classes of Plastics:

Propoxylated bisphenol-A furmarate polyester-styrene copolymer	.20	.20	.20

This amendment is effective November 30, 1970.

SANITARY ASPECTS OF MILKING SYSTEMS AND BULK FARM TANKS[1]

C. Bronson Lane[2]

Department of Animal Sciences
University of Kentucky
Lexington, Kentucky 40506

Abstract

The dairy industry has been a leader in protecting the consumer from pathogenic organisms in the milk supply. However, it does not always offer the consumer products with long shelf life, as is shown by keeping quality studies on dairy products obtained from retail outlets.

Both producers and processors can be blamed for the high incidence of dairy product dropouts, those products that are spoiled by the time they reach the consumer's refrigerator. Yet it must be emphasized that the finished product can be no better than the raw ingredient that went into the making of the product.

High quality milk can be produced by any dairyman having the will and motivation to succeed, the fixed determination to get the job done, and the proper training in sanitation principles.

Proper cleaning and sanitizing procedures for use on milking systems and bulk farm tanks are outlined. Proper sanitation procedures must be followed, as unclean milking equipment is a primary cause of quality problems on the farm and in the marketplace. With the full cooperation of our industry, dairy product dropouts will no longer plague the producers, processors, and ultimately, the consumers.

Dairy Product Dropouts—A Sanitation Problem

The dairy industry is by far the most regulated industry of any. Dairy-product consumers are very well protected in the areas of assured purity and nutritive value. There are more than 20,000 state, county, local, and municipal health and sanitation jurisdictions in the United States which assure a safe and wholesome milk supply. According to *Milk Facts (6)*, a recent U. S. Department of Agriculture study shows that milk plants are inspected on an average of 24 times a year!

To be sure, the dairy industry can take pride in the fact that it has been a leader in protecting the consumer from pathogenic organisms in the milk supply. But, has it protected the consumer from dairy product dropouts, those products that are spoiled by the time they reach Mrs. Housewife's refrigerator?

Langlois and Rudnick *(5)*, at the University of Kentucky, studied what the homemaker could expect in the way of keeping quality. Homogenized milk, skimmilk, low-fat milk, chocolate milk, half-and-half, and whipping cream were purchased from retail stores. The samples were placed in 40 F storage and checked for flavor on the 4th, 7th, 10th, and 14th days. Bacterial and taste analyses were made initially and after 7 days of storage.

A few shocking facts from this study will point up the severe shortcomings with dairy product keeping quality. Half of the whipping-cream samples failed to keep 7 days after purchase. Half-and-half was nearly as bad. Low-fat milks and skimmilk kept somewhat longer, but even some of these spoiled before a week was out. Homogenized milk had the lowest keeping quality. Almost 7% of the milk samples had a flavor score of less than 36 (edible, but not enjoyable) when brought into the laboratory for initial analysis.

Translating some of their findings into a consumer quality gamble, we learn that except for low-fat milks, the housewife has 1 chance in about 20 that the dairy product she buys won't be edibly enjoyable when she reaches home, and 1 chance in 8 that her milk won't keep more than 4 days in the refrigerator.

What's more, it has been shown repeatedly by extensive flavor surveys that 20% or more of the fluid milk on the market has recognizable off-flavors.

There is no doubt that both the producers and processors are responsible for this lamentable dairy product keeping quality. The point that must be emphasized, however, is the fact that the finished dairy product, be it homogenized milk, cream, or ice cream, is no better than the raw ingredient that went into making that product. Superior quality begins on the farm.

The Sanitation Perspective

The keeping quality of milk is dependent on its cleanliness, which in turn, depends upon the methods employed in its production and distribution. Hence, sanitary practices in milk production are of a paramount economic importance. Lack of keeping quality not only retards sales, but also causes the producer enormous losses because of premature souring or rejection of the milk by the plant processors. Then, too, Mrs. Housewife is more apt to try substitute dairy products if she purchases real dairy products with poor keeping quality. Therefore, in

[1]Presented at the 56th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, August 17-21, Louisville, Kentucky.

[2]Present address, Dairy Science Dept., U. of Florida, Gainesville, Fla.

perspective, proper sanitation spells profit

THE SANITATION PERSONNEL

In spite of the tremendous amount of educational work on sanitation carried out during the past decade, far too much milk has been rejected at the farm and in the marketplace. Someone has said that it is a strange anomaly that the milk producer, who has perhaps the greatest stake in the dairy industry, is, in general, the least concerned about the flavor and quality of the product he has to sell.

Perhaps this statement is not justified. It may be that those whose job it is to teach sanitary principles for milk production are not communicating properly with the producers. Today's "sanitation personnel" are well educated and trained. A dairyman has fieldmen, sanitarians, soap and sanitizer sales people, and university representatives at his disposal to help him produce a high-quality milk. He has probably been told how to get clean milk many times, but may never have been told why it is absolutely essential. Gayle Shrader, Chief of the Milk Control Section, Louisville and Jefferson County Health Department, has rightly said that enforcement should follow motivation and education. Too often, the reverse situation is in evidence. Clean milk can be produced by any dairyman having the will to succeed, the proper training and motivation in sanitation principles, and the fixed determination to get the job done.

PROPER SANITATION PROCEDURES

Much data has been published on the proper cleaning and sanitizing procedures to be used on milking systems and bulk tanks. This paper will not attempt to discuss the research in detail, but will merely point out basic sanitation principles to be employed for producing clean milk.

Cleaning and sanitizing milking systems

According to Barnard and Spencer *(3)*, pressure, vacuum, and vacuum-gravity systems are most commonly employed for circulation cleaning. The pressure method uses a centrifugal pump to push the cleaning solutions through the milking system. Large amounts of hot water and cleaners are necessary because the method uses a flooded system. Milking vacuum systems draw the solution through the units and the pipeline to the receiver jar, while air enters at the manifold to give velocity. The vacuum-gravity system is feasible on short lines only. The vacuum draws the solution to a tank at the far end of the line and the solution drains by gravity back to the sink through the same line.

Successful circulation cleaning of milking systems depends on the adoption of a complete cleaning program, based on cleaners and sanitizers correctly matched to the water supply. Obviously, an adequate supply of hot and cold water is essential for circulation cleaning. The water should be checked with a test kit or by a technical soap and sanitizer representative for mineral content, pH, and hardness. If the water tests more than 10 grains hardness, it should be softened or used with hard-water cleaners.

The IAMFES Dairy Farm Methods Committee *(8)* has reported that the four factors which are generally considered necessary for satisfactory circulation cleaning are: *(a)* adequate velocity, *(b)* a 5-min minimum circulation time with a 10-min optimum, *(c)* a solution of adequate strength, and *(d)* an end point temperature above 100 F. (Re-deposition of the cleaning solution and the minerals from water and milk will occur if the temperature drops too low.)

A general circulation cleaning procedure outlined by Arledge *(1)* and Barnard and Spencer *(3)* follows. Variations will occur with the brand of cleaner and sanitizer. Hence, the individual manufacturer's directions should be followed.

(a) Rinse line with tepid or lukewarm (100-120 F) water immediately after use. If possible, do not re-circulate, and continue rinsing until the water discharge is clear.

(b) Disassemble all parts which will not clean by circulation.

(c) Mix recommended amount of chlorinated cleaner with 160 F water.

(d) Re-circulate the wash water for 6-10 min, keeping it as near 140 F as possible. Brush all surfaces not cleaned by the solution.

(e) Drain wash water and rinse with acidified water. Drain and air dry. Plastic tubing more than 10 ft long should be dried with forced hot air.

(f) Just before milking, circulate a non-foaming sanitizer of proper strength.

Cleaning and sanitizing bulk tanks

Bulk tanks, if operating properly and efficiently, provide an inexpensive means of cooling and storing milk until picked up by the hauler for delivery to the processing plant. The use of bulk tanks continues to increase throughout the United States, according to the 14th annual Farm Milk Tank Survey, conducted by Dairy and Food Industries Supply Association. In January 1969, there were 233,563 tanks in use compared with 229,827 in January 1968. In 1964, there were less than 200,000 bulk milk tanks in operation.

Bulk tanks have eliminated some problems, but they have introduced others. Atherton *(2)* states that the tendency toward longer storage, greater transport distances, and increased refrigeration for raw milk have given an opportunity for a group of cold-loving organisms to gain tremendous importance in milk quality control.

These bacteria, known as "psychrophiles," can grow fairly rapidly at cooler (38-40 F) temperatures and can cause spoilage of milk which results in such flavor criticisms as flat, bitter, stale, ropy, fruity, putrid, and unclean. While psychrophiles grow at low temperatures, they grow faster at higher temperatures.

The main sources of psychrophilic bacteria are soil and water, but they are also found to a significant degree in dirty equipment and raw milk. Many of these bacteria are both proteolytic and lipolytic, and most are at least proteolytic. Organisms seen as psychrophilic include: *Pseudomonas, Aerobacter, Alcaligenes, Escherichia, Flavobacterium, Achromobacter, Proteus, Streptococcus, Micrococcus,* and *Chromobacter.*

To prevent psychrophilic spoilage of raw milk, it is imperative that the bulk tank unit be cleaned and sanitized properly. Then, too, it must be operating properly and have an adequate cooling capacity. Even though the psychrophiles grow fairly well at temperatures below 40 F, growth of the majority of bacteria found in raw milk is very slow in this temperature range. However, it doesn't take long for certain bacteria to multiply and exceed acceptable standards for bacterial numbers (100,000 per milliliter Standard Plate Count for Grade A raw milk) at higher temperatures, according to Randolph *(7).*

To keep bacterial contamination to a minimum, it is recommended that the following procedure be used for hand cleaning and sanitizing bulk tanks:

(a) Rinse bulk tank with tepid water.

(b) Brush-wash inside of the tank with recommended amount of alkaline cleaner (chlorinated-foaming type) mixed with 160 F water in pre-measured pail or wash vat. After the tank, bridge, agitators, covers, and other parts have been washed, drain solution from the tank. The valve can be brushed while the solution is draining. The same solution can be used on the outside of the tank.

(c) Rinse residual detergent from tank, then follow with a cold-water acid rinse (if necessary).

(d) Sanitize prior to placing milk in the tank.

(e) Look! Make sure that the tank is clean before using again. Pay particular attention to the outlet valve, the under-fixed portions of the top of the tank, and under the blades of fixed agitators.

This same procedure can be used on pail milkers and utensils with a 120 F wash water.

Inevitably, more and more farm bulk tanks are being equipped for mechanical cleaning. Some have adjustable agitators which can be speeded up to spray prepared cleaning solution over the entire tank surface. Others are equipped with spray devices. These systems require substantial amounts of water and detergent. Non-foaming cleaners must be used to prevent serious rinsing problems.

The following cleaning and sanitizing procedure is recommended for bulk milk tanks equipped with mechanical cleaning equipment:

(a) Rinse bulk tank thoroughly with tepid water as soon as the milk is removed.

(b) Fill tank with 150 F water to cover outlet valve or agitator pickup "blades." Add recommended amount of chlorinated non-foaming cleaner.

(c) Start mechanical cooling device and operate for approximately 10 min. Drain the cleaning solution and brush-wash valve.

(d) Rinse tank with acidified cold water.

(e) Sanitize prior to placing milk in the tank.

The Klenzade Dairy Farm Sanitation Handbook (4) states that the cleaning and sanitizing procedure can be effective only if it is carried out after each milk pickup.

Improper cleaning of milking equipment will cause the deposition of films. Milkstone or waterstone films, which are white or yellow, are caused by hard water. Proper cleaning procedures with products that are compatible with the water supply will prevent mineral deposits. Concentrated acid cleaners can check this film.

Protein deposits, which take on a bluish-gray color as they become thick, are caused by inadequate rinsing, low-solution temperatures, weak cleaning solutions, and irregular cleaning, and can be removed with a chlorinated alkaline cleaner. Fat films are indicated by hanging water droplets or a greasy surface, and are caused by weak solutions, irregular washing, low-solution temperatures, and inadequate rinsing.

ULTIMATE SANITATION POTENTIAL

Proper sanitation procedures must be followed, as unclean milking equipment is a primary cause of quality problems, on the farm and in the marketplace. We have the necessary technological information on cleaning and sanitizing to insure a clean, safe, and wholesome milk supply. The producer must

be educated and motivated to use it. With the full cooperation of our industry, dairy product dropouts will no longer plague the producers, processors, and ultimately, the consumers.

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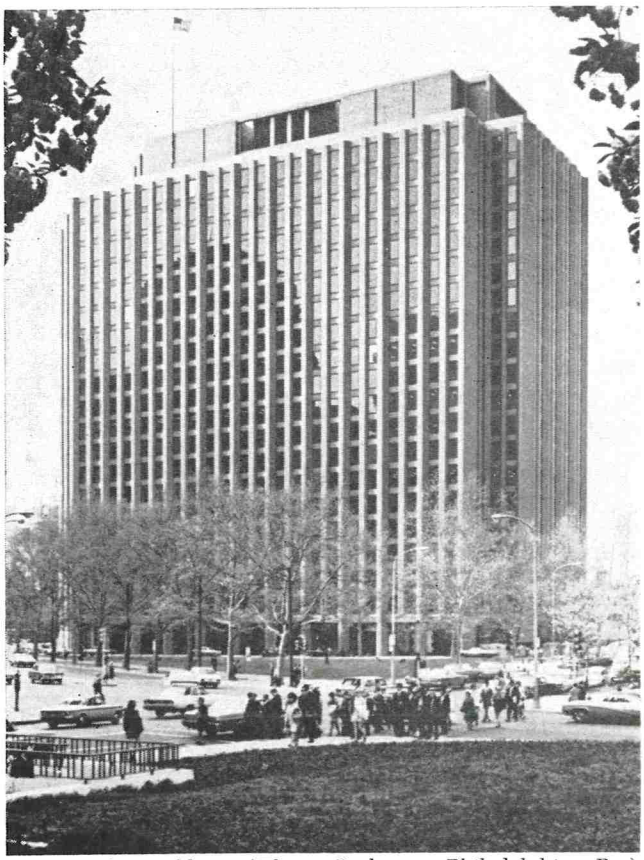
NEWS & EVENTS

PENNWALT MOVES INTO NEW HEADQUARTERS—ITS OWN 20-STORY BUILDING IN PHILADELPHIA



Pennwalt Building (Three Parkway, Philadelphia, Pa.), company's new corporate headquarters.

Pennwalt Corporation has moved into its new headquarters building at Three Parkway, Philadelphia. A distinctive 20-story brick structure boasting a number of design and service innovations, it is known as the Pennwalt Building and is located between 16th and 17th Streets on the north side of the picturesque Benjamin Franklin Parkway.

The move into its own building further centralizes corporate headquarters in a new location for Pennwalt's worldwide operations. In addition to departments and divisions formerly located at Three Penn Center, personnel from other locations also moved into the new building.

100TH CLASS IN BAKING SCIENCE AND TECHNOLOGY TO BE PRESENTED

The American Institute of Baking's 100 course in Baking Science and Technology will be presented August 2 - December 17, 1971. The dates of the anniversary course were announced in the 1971 schedule of classes issued recently.

Expanded information within the established framework of classes will mark the 1971 AIB offerings. The schedule provides for the seven courses presented in 1970 to be offered again in 1971. Each course, according to Temple R. Mayhall, director of education, will be even more concentrated as the AIB attempts to provide students with the most complete and up-to-date baking and sanitation information available.

The new schedule incorporates the tuition increases authorized by the Board of Directors in March. The fees include all class materials, books, etc. necessary for the course.

The first 20-week course in Baking Science will be offered January 23 - June 11, 1971. The deadlines for scholarship applications are December 1, 1970 for the January course and June 1, 1971 for the August course.

Baking for Allied Personnel, a two-week course, will also be presented twice - January 11 - 22 and July 19 - 30.

The other courses scheduled for 1971 are: Basic Principles of Equipment Maintenance, February 8 - 26; Engineering and Maintenance Technology of

Equipment (Bakery Equipment Engineering), September 13 - 17; and Food Plant Sanitation, November 1 - 5.

Additional Information and application forms are available from the Registrar, American Institute of Baking, 400 E. Ontario St., Chicago, Ill. 60611.

VETERAN INSTRUCTOR RETURNING FOR SPECIAL COURSE

Byon O. "Ben" Norton, AIB bread instructor from 1936 until his retirement September 1, 1968, is returning to the Institute as an instructor for a special course November 30-December 11, 1970.

According to Temple R. Mayhall, director of education, Mr. Norton will be responsible for the students' experimental bake shop work. Although the special class will run concurrently with the 20-week course in Baking Science and Technology, there will be no interference with the regularly scheduled shops.

Ben Norton taught bread production to 57 classes during his tenure at the AIB. During World War II, he and other school personnel organized and served on the faculty of the Army Quartermaster Corps' Advanced School of Baking at Camp Lee, Virginia.

Mr. Norton joined the School of Baking faculty upon graduation from the course in Baking Science and Technology, then 16 weeks. The curriculum, expanded to 20 weeks, has grown to include sweet goods, sanitation, maintenance, safety and personnel problems.

He was succeeded as bread instructor by James A. Boge.

BOOK ON CHEESE AND FERMENTED MILK FOODS

A 3rd printing of the popular book, *Cheese and Fermented Milk Foods*, is now available through Edwards Brothers, Inc., Box 1007, Ann Arbor, Michigan 48106. Published four years ago, the volume has been accepted by industries and schools in more than 50 countries.

The original 437 page book, richly illustrated with photographs and pen and ink sketches, showed the principles and manufacturing steps of 60 cheeses and fermented milk foods, including cottage, Cheddar, Mozzarella, and cream cheeses, and yoghurt, buttermilk, and sour cream. It also included practical aspects of cheese safety, nutrition, and economics.

The new printing contains improvements and revisions including the updating of key statistics, new material on fruit-flavored buttermilk and yoghurt, and trends in mechanizing of Italian soft cheese.

Little known facts like the identity of the manufacturer of domestic Kefir grains, the movement away from pasteurized milk for Cheddar cheese, and the caloric levels of yoghurt add to interest and make the 3rd printing of the book valuable as a reference.

JOHN L. SADOWSKI

The untimely death August 19th of John L. Sadowski has robbed St. Louis citizens of one of their most dedicated public servants. Mr. Sadowski, as Deputy Health Commissioner, was engineering director of the Environmental Sanitarian Services, covering such diversified Public Health fields as Food, Milk, Meat, Mosquito and Rat Control, as well as general sanitation. He was the guiding, driving force behind the effort that brought hundreds of thousands of dollars of federal funds into St. Louis to enable the Health Division to intensify its efforts in the control of rat infestation.

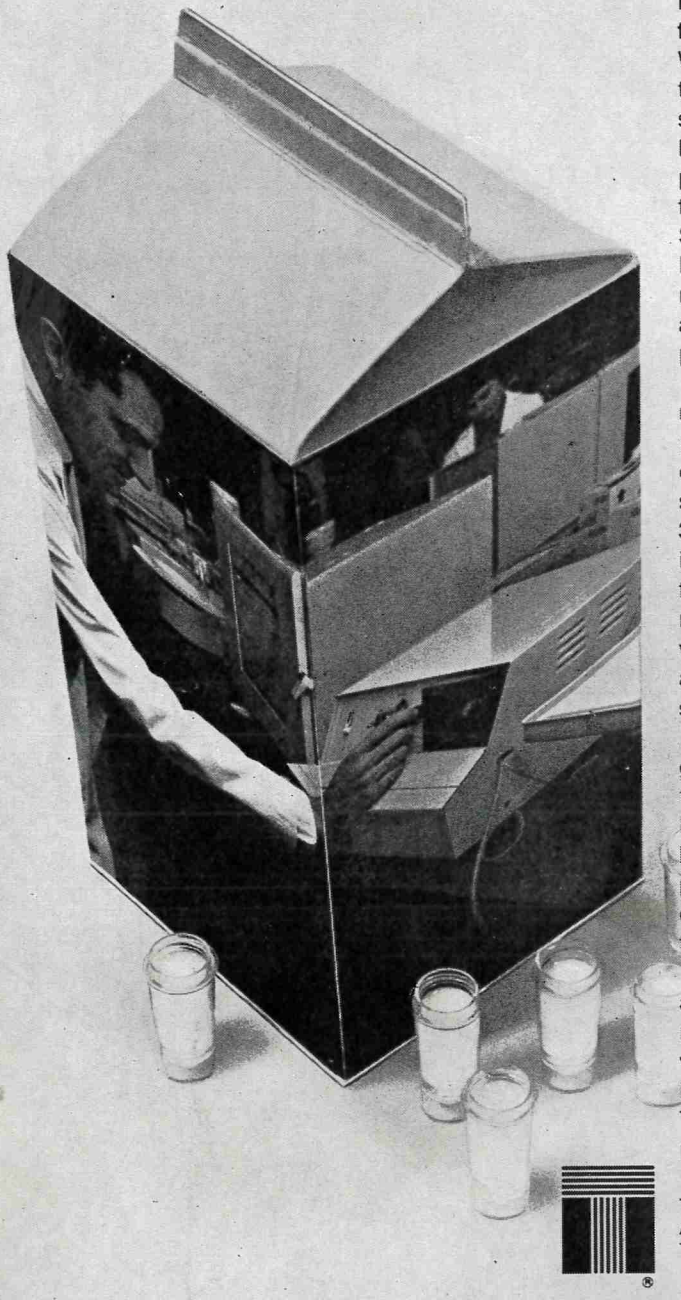
Mr. Sadowski's association with the St. Louis Health Division goes back more than 30 years, from the late 1930's when he became a field engineer in the Milk Control Section following his graduation from the University of Missouri with a degree in chemical engineering, to his appointment as Deputy Health Commissioner in 1964, the position he held at the time of his death.

During World War II he saw service with the United States Army Corps of Engineers and the United States Public Health Service in the field of safety and sanitation of water supplies throughout the United States.

In 1947 the St. Louis Health Division established the citys' first Rat Control Section, with John Sadowski as its Chief. Rat Control at that time was in its infancy, and many of the rat-stoppage and rat eradication theories and methods now accepted as common policy all over the nation were initiated and developed in St. Louis under Mr. Sadowski's supervision and guidance.

In 1951 Mr. Sadowski left the city's service to accept a position in private industry, but returned to the Health Division as Chief of Rat Control in 1955, and remained in that capacity until his appointment as Chief of Environmental Sanitation Services.

The New Technicon® Somatic Cell Counter



Faster and more reliable testing for abnormal milk

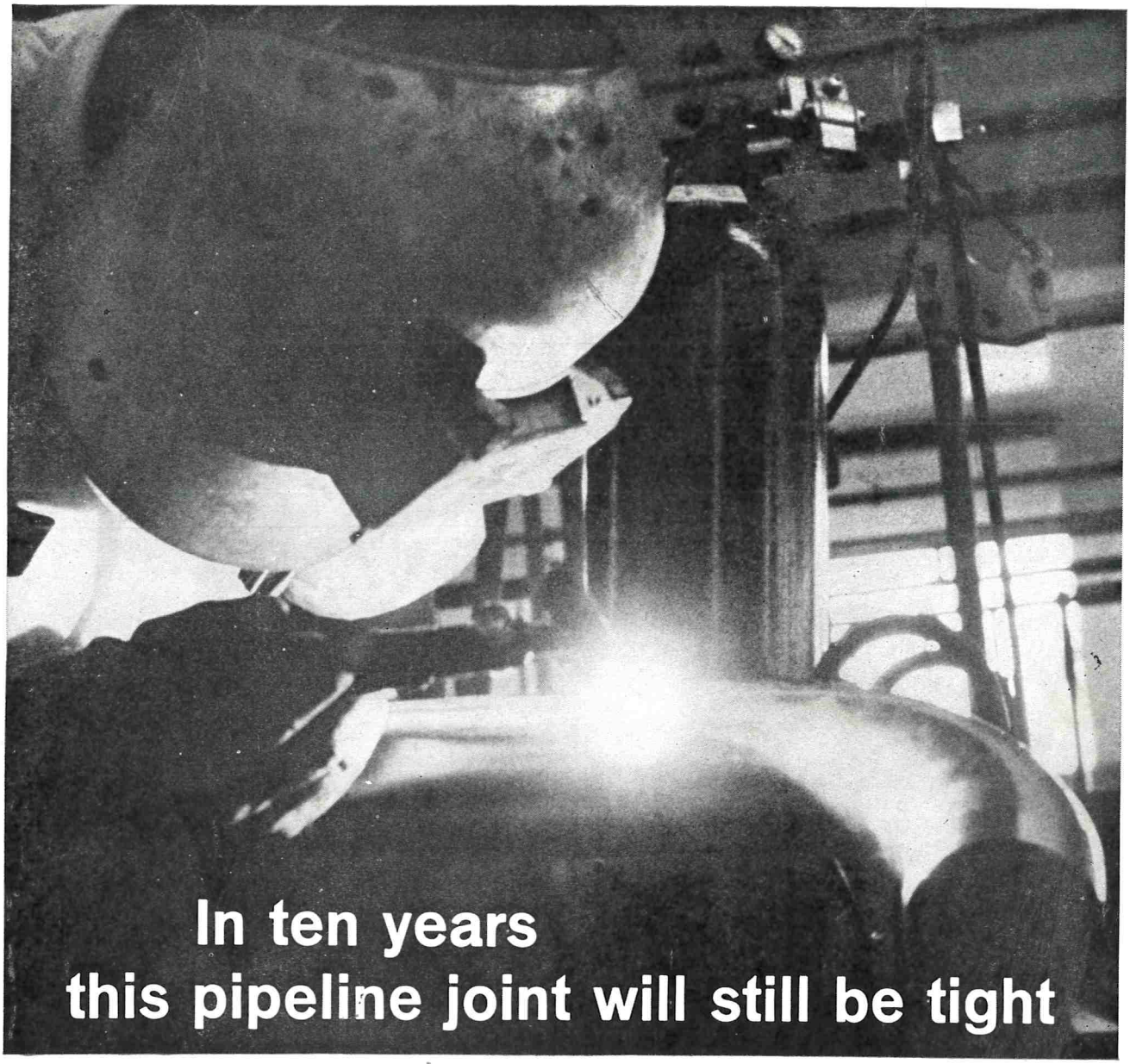
We probably don't have to tell you about the new regulations now in effect concerning somatic cells. But we do want to let you know how we can help you test more samples, which is the immediate problem that these regulations present. With the new Somatic Cell Counter from Technicon your laboratory can do from three to five times as many samples as are done manually without adding any new laboratory space or personnel.

The first *fully* automatic system on the market, the Technicon Somatic Cell Counter performs an accurate count on milk samples on a continuous, unattended basis, at a screening rate of 60 samples per hour. It does 30 samples per hour for confirmatory counting, and both the screening and confirmatory tests can be run automatically. The automatic operation includes sample processing, which makes manual preparation, screening, and counting a memory, and routine quarter sampling an everyday reality.

Technicon also makes it easier for you to get a new unit into your laboratory by making the Somatic Cell Counter available on a sale, lease, or "try it before you buy it" trial lease basis. The unique 4-month Trial Lease makes possible the application of a good portion of the monthly fee to future purchase of the system.

Technicon also makes your conversion to automation easy by supplying complete reagent and service contracts, and by providing a free training course in the use of the instrument. For further information on how Technicon® Systems can help you, please write to Department 110:

Technicon Industrial Systems
A Division of Technicon Instruments Corporation
Tarrytown, New York 10591



No leaks — easily cleaned — maintenance free permanent — welded by Surge

Welded milk lines are available from your Surge Dealer, wherever you may dairy in the United States or Canada. A concept proven in milk processing plants and on the dairy in localized areas for more than 15 years is now offered by Surge Dealers everywhere.

Expansion and contraction of the milk line along with gasket aging in conventional couplings can cause cracks and leaks. Cracks represent contamination sources and leaks are detrimental to milk quality and efficient milking. Welded lines can eliminate these problems.

All welded lines installations are subject to the approval of local regulatory authorities.

Thorough, efficient cleaning, which results in a higher quality milk product, is of interest to everyone — dairymen, sanitation specialists, dairy plant fieldmen, and consumers.

SURGE...the accent is on YOU

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