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FACTORS ASSOCIATED WITH THE DURATION OF GESTATION IN DAIRY CATTLE

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The duration of gestation in dairy cows is of importance to the dairy husbandman from a management standpoint, for it is desirable that dairy cows be in a non-lactating state during the last 6 to 8 wk. of each pregnancy. Thus, it is possible to prepare the cow physically for parturition and her next lactation. Dairy cattle breed associations also require that a living calf be produced within a maximum time limit after the completion of certain types of official production records. The purpose of this requirement is to assist in verifying the duration of pregnancy during the official testing period. Recently the Brown Swiss Cattle Breeders' Association has extended the calving requirements following the completion of an official record to conform with the abnormally long gestation period found to exist in the Brown Swiss breed. Likewise, the Holstein-Friesian Association has reduced the gestation length used in the verification of applications for registration. This decision was based on the preliminary results of this study and data from similar investigations. In addition to these very practical applications, the possibility of various environmental and genetic factors influencing the duration of gestation is intriguing and may lead to fundamentally important discoveries.

The commonly accepted opinion at the beginning of the nineteenth century was that normal gestations for dairy cattle averaged 270 days (33). Systematic investigations soon revealed this figure to be less than that normally observed. In 1817, Tessier (8) is reported to have found that 575 gestations averaged 282.2 days. Then, in 1840, the Earl of Spencer (33) is reported to have made a detailed study of Durham gestations. His results showed the average length of 764 gestations to be 283.3 days. The range for calves born alive, reported by Spencer, was from 220 to 313 days. However, no calves born earlier than 242 days lived. Over half a century ago, Fleming (18) reported the average gestation of cattle to be 283 days. Of the 1,062 observations included in his summary, 15 gestations were less than 241 days and 32 greater than 301 days.

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During the present century, numerous studies have been made in an attempt to determine if various factors were related to gestation length in dairy cattle. Considerable difference of opinion apparently exists. For instance, Williams (58) states that any material deviation from the typical physiological duration of pregnancy is indicative of pathological influence. Therefore, the results of previous investigations are reviewed in the respective sections of this report. In this study, an attempt was made to determine whether or not an association existed between gestation length in dairy cattle and the following factors: (a) breed, (b) sire, (c) time dam spent *in utero,* (d) age of dam, (e) sex of fetus, (f) birth weight of calf and (g) season of year.

A. INTER-BREED DIFFERENCE

McCandlish (45), whose investigation included all the major dairy breeds except Brown Swiss, concluded that length of gestation was not affected by the breed. In studies which included only the Brown Swiss breed (15, 16, 25, 26, 55), mean duration of pregnancy has ranged from 289 to 291 days (table 1). These extensive data concerning Brown Swiss indicate that the average gestation length of this breed exceeds the average of dairy and beef breeds which has been found to be 282.1 days (43). Henderson (22), whose investigation included the five major breeds of dairy cattle and Shorthorns, substantiates this opinion. He found that Brown Swiss were the only breed whose mean gestation length was significantly different from that of the other breeds. Additional evidence concerning the existence of a breed difference among cattle in the mean duration of pregnancy has been furnished by Rife *et al.* (49), who found the difference between the mean gestation length of Aberdeen-Angus and Herefords to be highly significant. A highly significant difference in the length of Aberdeen-Angus and Hereford gestations likewise was reported by Johnson (29). Johnson further found that the difference between Aberdeen-Angus and Shorthorn gestations was significant. An investigation by Livesay and Bee (41) revealed that the difference between the mean gestation length of the beef breeds and the dairy breeds was highly significant. They, too, found a highly significant difference between the mean gestation length of Aberdeen-Angus and Herefords. Littlewood (40) reported that the mean gestation length of Ongole was significantly different from the means of Kangayam and Sind. Recently Alexander (2) has reported that gestations of the Brown Swiss and Guernsey breeds were distinctly longer than the accepted average of 281 days and the mean of each of the other major dairy breeds were distinctly below this average.

TABLE 1

Duration of gestation in cattle and related species

Breed	Author[a]	Year reported	No. observed	Average length
				(*d.*)
Ayrshire	Livesay (41)	1945	580	277.8
Ayrshire	McCandlish (45)	1922	37	278
Ayrshire	Alexander (3)	1950	311	278.2
Ayrshire	Henderson (22)	1938	130	280.4
Ayrshire	Fitch (17)	1924	113	284.6 ± 0.4

Breed	Author[a]	Year reported	No. observed	Average length
				(*d.*)
Brown Swiss	Henderson (22)	1938	38	287.4
Brown Swiss	Engeler (16)	1948	3,000	289.0
Brown Swiss	Blum (16)	1948	3,838	289.1
Brown Swiss	Alexander (3)	1950	168	289.6
Brown Swiss	Idtse (25)	1948	448	289.8
Brown Swiss	Zwicky (16)	1948	1,000	290.0
Brown Swiss	Ineichen (26)	1946	3,105	290.9 ± 0.1
Brown Swiss	Weaver (55)	1947	148	291
Guernsey	McCandlish (45)	1922	125	281
Guernsey	Henderson (22)	1938	120	281.8
Guernsey	Fitch (17)	1924	103	283.0 ± 0.5
Guernsey	Herman (23)	1947	119	284.0
Guernsey	Alexander (3)	1950	277	285.1
Friesian	Jordao (30)	1943	161	276.2
Friesian	Veiga (53)	1947	1,130	276.2 ± 0.4
Holstein-Friesian	McCandlish (45)	1922	111	278
Holstein-Friesian	Herman (23)	1947	962	278.1
Holstein-Friesian	Knoop (35)	1934	432	278.2 ± 0.2
Holstein-Friesian	Livesay (41)	1945	415	278.3
Black Pied	Dinkhauser (12)	1944	311	279.7
Holstein-Friesian	Henderson (22)	1938	437	279.8
Holstein-Friesian	Knott (36)	1932	2,824	279.9 ± 0.06
Holstein-Friesian	Wing (59)	1899	97	280
Holstein-Friesian	Alexander (3)	1950	666	280.4
Holstein-Friesian	Fitch (17)	1924	220	281.0 ± 0.2
Friesian	Hewitt (24)	1934	123	282
Jersey	Livesay (41)	1945	265	277.9
Jersey	Copeland (8)	1930	1,075	278.5
Jersey	Henderson (22)	1938	179	278.8
Jersey	Knoop (35)	1934	373	278.9 ± 0.2
Jersey	McCandlish (45)	1922	92	279
Jersey	Wing (59)	1899	56	279
Jersey	Alexander (3)	1950	300	279.6
Jersey	Herman (23)	1947	876	280.4
Jersey	Fitch (17)	1924	100	284.3 ± 0.7
Aberdeen-Angus	Long (42)	1948	99	276.4
Aberdeen-Angus	Johnson (29)	1944	112	280.9
Aberdeen-Angus	Livesay (41)	1945	173	282.5
Hereford	Paim (48)	1944	.....	279.3 ± 1.3
Hereford	Johnson (29)	1944	98	283.4
Hereford	Livesay (41)	1945	174	285.2
Hereford	Long (42)	1948	101	286.3
Red Poll	Hewitt (24)	1934	788	285
Shorthorn-Beef	Knapp (34)	1940	164	280.8
Shorthorn-Beef	Sabatini (4)	1908	120	280.9 ± 0.1
Shorthorn-Beef	Dawson (10)	1947	307	281.2
Shorthorn-Durham	Spencer (33)	1840	764	283.3
Shorthorn-Beef	Johnson (29)	1944	34	283.5
Shorthorn-Milking	Knapp (34)	1940	133	281.7
Shorthorn-Milking	Henderson (22)	1938	30	281.8
Bernese	Jakubec (27)	1941	.....	290.1
Caracu	Jordao (31)	1938	980	286.9 ± 0.4
Hungarian	Wellman (56)	1910	228	284.6
Mocho	Jordao (32)	1939	278	286.5 ± 0.6
Montafon	Jakubec (27)	1941	.....	281.5
Montafon	Ogrizek (46)	1939	.....	284.6 ± 0.8
Simmental	Ogrizek (46)	1939	.....	286.2 ± 0.5
Simmental	Wellman (56)	1910	291	291.2
Swedish Red and White	Johansson (28)	1928	10,219	283.7 ± 0.06
Nellore	Veiga (52)	1946	254	291.4 ± 0.5
Ongole	Littlewood (40)	1937	.....	289.2
Sindhi	Dave (9)	1934	.....	283
Carabao	Gonzales (19)	1919	3	323
Water Buffalo	Levine (39)	1920	2	312
Yak	Denisov (11)	1938	.....	258

[a] For the purpose of brevity, the names of only the senior authors are used in this table.

Results of these investigations and others which have contributed information concerning the mean gestation length of cattle and related species are summarized in table 1 to facilitate their comparison. Breed averages have not been calculated because differences have existed in the basis for the selection of gestations used in the various studies. For instance, Knott (36) excluded all gestations which had been recorded as abnormal on the breeding records. Others (23, 34, 55) excluded all gestations which were classified as abortions. Knoop and Hayden (35) included only those gestations ranging from 264 to 297 days and Henderson (22) confined his study to those gestations within the range of 265 to 295 days. Further, as will be explained later, a uniform procedure has not been used by the investigators in calculating the number of days in these gestations.

PROCEDURE

The data used in this study were compiled from records which have been carefully kept on the Ohio State University purebred dairy herd from 1922 to 1949. Each of the gestations terminated by the birth of a living, single, normal-appearing calf was included, regardless of the length of gestation, to determine the average duration of pregnancy for each of the five major dairy breeds included in this herd.

The gestation period in cattle is from the time of fertilization of the ovum to parturition. However, due to the impossibility of determining the exact time of fertilization of the ovum, the last service date is used instead of the time of fertilization (43). The previously mentioned difference concerning the calculation of gestation length consists of whether one or both terminal dates are counted. Fitch *et al.* (17), Knott (36) and White (57) included both the day the animal was served and the day the calf was dropped. Copeland (8) and Knoop and Hayden (35) included the day the calf was born but not the day of service. Many of the other investigators have not indicated which method was followed. In the computation of data for vital statistics, only one of the two terminal dates is included (60). Therefore, in calculating the number of days in the gestations used in this study, the day the calf was born has been counted but not the day of last service.

In compiling the data for this study, it was observed that four gestations of less than 200 days were terminated by the birth of living, normal calves. These gestations were 149, 169, 183 and 196 days when computed from the last service date. However, upon using service dates prior to the last, the above gestations were found to be 280, 278, 289 and 283 days, respectively. The first two of these four cows each appear to have had one post-conception service. Further, the third cow must have had two and the fourth cow four post-conception services. In addition to these four cows, there were 26 other gestations ranging from 201 to 265 days when computed from the last service dates. Assuming conception to have occurred prior to the last service dates, these gestations then have a range of 272 to 294 days in length. Of these 26 gestations, one post-conception service apparently occurred in 24 of the cases and two post-conception services in the other two. Each of these 30 gestations had been terminated by the birth of a

living, single, normal calf, so it is highly probable that conception occurred at services prior to the last service. Therefore, in computing the length of these 30 abnormally short gestations, the last service dates were disregarded and earlier service dates giving a more nearly normal length gestation were used. Post-conception services have been reported in cattle by Donald (13) and Knapp *et al.* (34). Ward and Castle (54) and Asdell (3) have suggested the possibility of post-conception services in cattle and sheep, respectively. The occurrence of estrus during pregnancy is a result of less-than-normal suppression of cyclic changes (20).

In other studies (2, 5, 6, 8, 12, 17, 23, 24, 26, 27, 28, 36, 46, 47, 54, 55, 59) the average length of gestations terminated by the birth of twins has been found to be from 1.6 to 12 days less than that for single births. Usually, in those studies which included the larger numbers of gestations, the difference was found to be from 3 to 6 days less. In view of this difference, only gestations terminated by the birth of single calves were included in this study.

RESULTS AND DISCUSSION

The 1,256 gestations terminated by the birth of living, single, normal calves averaged 279.58 days in length. The range was from 245 days for a Jersey to 305 days for an Ayrshire (table 2). In the preceding gestation this Jersey cow

TABLE 2

Frequency distribution of gestation lengths

Class interval	Ayrshire		Brown Swiss		Guernsey		Holstein-Friesian		Jersey	
(*d.*)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)
Below 250	0		0		0		0		1	0.4
250 to 254	0		0		0		0		1	0.4
255 to 259	0		0		0		0		0	
260 to 264	3	0.9	0		0		2	0.6	1	0.4
265 to 269	4	1.2	0		0		13	3.7	12	4.8
270 to 274	46	13.9	0		10	3.6	49	13.8	49	19.7
275 to 279	164	49.6	1	2.2	58	20.9	149	42.1	91	36.6
280 to 284	100	30.2	6	13.3	116	41.9	94	26.5	69	27.7
285 to 289	12	3.6	21	46.7	74	26.7	37	10.4	18	7.2
290 to 294	0		16	35.6	16	5.8	8	2.3	6	2.4
295 to 299	1	0.3	1	2.2	2	0.7	2	0.6	1	0.4
300 and over	1	0.3	0		1	0.4	0		0	

had aborted at 5 mo. However, this calf born at 245 days lived and the records, which in this and many other cases do not include birth weights, give no indication of any abnormality in the calf. The other Jersey calf born following a 253 days gestation (table 2) was not related to the previously mentioned calf. The two Ayrshire calves carried *in utero* for 296 and 305 days were unrelated.

In this paper, differences referred to as significant are those which due to their magnitude can be expected to happen by chance alone in 5 to 1 per cent of the trials. In like manner, highly significant differences are those which on the basis of chance alone could be expected in less than 1 per cent of the trials. A comparison of the breeds in table 3 shows that the smaller variances are found

TABLE 3

Duration of pregnancy in gestations terminated by birth of living, single calves

Breed	No. of gestations	Av. no. of days	Variance	Standard error
Ayrshire	331	278.16	18.23	0.23
Brown Swiss	45	288.36	16.50	0.61
Guernsey	277	282.67	22.02	0.28
Holstein-Friesian	354	278.57	28.44	0.28
Jersey	249	277.87	35.40	0.38
Total	1,256	279.58		

in the Brown Swiss and Ayrshire breeds. Using Bartlett's test of homogeneity of variance (50), the difference between these variances was found to be highly significant, giving a Chi-square of 41.17 with 4 degrees of freedom. The possibility of genetic influence on gestation length is suggested by the fact that, in this herd, line breeding has been practiced much more in the Brown Swiss and Ayrshire breeds and the variances of gestation length are less for these two breeds than for the other breeds.

The significance of the difference between the means of each breed from that of each of the other breeds was tested by the approximate method of testing the hypothesis, $m = o$, with no hypothesis about the variance (50). Table 4 shows the means of both the Brown Swiss and Guernsey gestation lengths to be significantly different from those of each of the other breeds at the 1 per cent level. The differences between the mean gestation lengths of the Ayrshire, Holstein-Friesian and Jersey breeds are not significant. These results are contrary to McCandlish's (45) conclusion regarding breed differences and the mean gestations found for the Ayrshire and Jersey breeds are considerably less than those reported by Fitch *et al.* (17). However, the mean durations of pregnancy for each of the breeds in this study are in general agreement with those reported by most of the recent investigators (table 1). The fact that no study has shown a significant

TABLE 4

Significance of the difference between the means of gestation length

Breeds	Difference between means	Standard error of difference	t[a] value	$t_{0.05}$[a]	$t_{0.01}$[a]
	(*d.*)				
Brown Swiss-Ayrshire	10.20	0.649	15.70**	.....	2.68
Brown Swiss-Guernsey	5.69	0.668	8.52**	.....	2.68
Brown Swiss-Hol.-Friesian	9.79	0.669	14.46**	.....	2.67
Brown Swiss-Jersey	10.49	0.713	14.70**	.....	2.67
Guernsey-Ayrshire	4.51	0.367	12.29**	.....	2.59
Guernsey-Hol.-Friesian	4.10	0.400	10.26**	.....	2.59
Guernsey-Jersey	4.80	0.471	10.19**	.....	2.60
Hol.-Friesian-Ayrshire	0.41	0.368	1.11	1.98	.....
Hol.-Friesian-Jersey	0.70	0.472	1.48	1.97	.....
Ayrshire-Jersey	0.29	0.444	0.65	1.97	.....

[a] Approximate t value when testing the hypothesis, $m = o$, with no hypothesis about the variance (50).

** $P < 0.01$.

difference to exist between the gestation lengths of Holstein-Friesians and Jerseys indicates that within species there is little or no association between gestation length and either body size or rate of sexual maturity. Likewise, in horses gestation length and body size have been found not to be related (3). It is interesting to note the apparent similarity of duration of gestation in Brown Swiss and Indian cattle. This similarity is of particular interest when considering other similar sexual characteristics, such as external signs of heat and duration of heat period. Asdell (4) states that the mean duration of heat for Indian cattle is 4.8 hr. compared to 13.6 hr. for European breeds. He also mentions that external signs of heat are practically unrecognizable in Indian cattle. The senior author has observed that Brown Swiss, when compared to other European breeds in the same herds, commonly are considered to have a shorter duration of heat and to show less pronounced external signs of heat. Additional study on this topic could possibly establish an association between these characteristics and gestation length. Hammond (20) and Snyder (51) have suggested that a relationship exists between the variation in the length of the estrous cycle and gestation length.

Gestations terminated by the birth of twins have not been included in the above data. During the period included in this study, the 37 gestations terminated by the birth of twins, either living or dead, averaged 274 days. The 30 gestations terminated by the birth of twins in which one or both calves were alive averaged 276.23 days or 3.35 days less than the gestations terminated by the birth of live, single calves.

B. INTER-SIRE DIFFERENCE WITHIN BREEDS

Reid is reported to have observed in 1850 that the sire appeared to have some influence on the gestation length in the cow (33). Knott (36) also recognized the possibility of paternal influence on the duration of gestation. As a result of cross-breeding Aberdeen-Angus and Herefords, Rife *et al.* (49) concluded that the sire was a potent influence in determining the duration of pregnancy because, irrespective of the breed of the sire, the mean gestation lengths of the cross-bred offspring were almost intermediate between those of their parents. The differences between the means of each of the parents and those of the crossbreds were highly significant. Alexander (2) and Bonadonna *et al.* (6) both observed distinct differences in the time progeny of different sires spent *in utero*. Jordao and Viega (31, 32) have substantiated these observations by finding significant inter-sire differences within breeds.

RESULTS AND DISCUSSION

From the data used in the preceding part of this study, the five sires responsible for the largest number of gestations in each of the Guernsey, Holstein-Friesian, Jersey and Ayrshire breeds were selected. Brown Swiss were not included because both the number of sires used and the number of cows mated were considered to be too few to produce worthwhile evidence. The range of the mean gestation lengths per sire within each of the breeds is shown in table 5. The inter-sire difference within both the Guernsey and Holstein-Friesian breeds

was found to be significant at the 1 per cent level when the analysis of variance (50) was used. The inter-sire difference was not significant in the Ayrshire breed where more line breeding had been followed.

These results indicate that the length of gestation is influenced by the genotype of the fetus and not determined entirely by its environment. The effect of the genotype of the fetus on gestation length can be explained by the fact that both parturition and its inhibition are under hormonal control (51) and that the fetal portion of the placenta probably has hormonal functions during pregnancy (7).

C. ASSOCIATION BETWEEN TIME THE DAM AND HER PROGENY SPENT IN UTERO

Two or more gestations of the same cow may vary widely in length. Copeland (8) found no correlation between the length of pregnancies for the same dam. However, in spite of this intra-dam variation, some investigators (28, 31, 34, 36, 59) have observed that many individual cows had a characteristic length of gestation. Knapp *et al.* (34) found the variation between cows to be highly

TABLE 5

Significance of inter-sire difference in gestation length within breeds

Breed	Range in no. gestations per sire	Range in mean gestation lengths/sire	F value (Analysis of variance)	$F_{0.05}$
Guernsey	37–44	280.7–285.5	5.79**	2.41
Holstein-Friesian	29–61	278.4–281.7	3.65**	2.41
Jersey	25–36	275.3–280.2	2.79*	2.43
Ayrshire	29–75	277.0–279.0	1.75	2.41

* $P > 0.01 < 0.05$
** $P < 0.01$

significant. Johansson (28) found a positive correlation of 0.29 ± 0.02 between the second and third gestations for the same cow. Knott (36) observed, in four of the 11 cows studied, a similarity in the length of time a cow carried her calves and the time she had spent *in utero*. Such associations could result from the dam's combined genetic and environmental influence on the fetus or from similar environmental influences during the two gestations.

RESULTS AND DISCUSSION

The correlations between the time the dam spent *in utero* and the time her individual progeny were carried were based upon 100 Ayrshire and 100 Jersey gestations which had been selected in an unbiased manner from those used in the first part of this study. However, this selection was restricted to the three sires in each breed with the largest number of progeny. The correlation coefficient of + 0.32 for the Ayrshires was highly significant. Within the Jerseys the correlation of + 0.20 was significant but less so than within the Ayrshires where line breeding had been practiced. If line breeding was responsible for increasing this association, it is an indication of genetic influence.

D. EFFECT OF AGE OF DAM UPON GESTATION LENGTH

Previous investigators are not entirely in agreement regarding the effect of the age of dam upon gestation length. For instance, Copeland (8), Jakubec (27), Knapp *et al.* (34), McCandlish (45) and Weaver *et al.* (55) found no definite trend in length of gestation between dams of different ages; another group consisting of Herman and Spalding (23), Ineichen (26), Johansson (28), Knoop and Hayden (35) and Knott (36) found differences ranging from 1 to 3 days between the mean gestation lengths of 2-yr.-old dams and mature cows. In some of these studies a decrease in gestation length was noted after the cow passed the age of 9 yr. but this trend was less definite than the increase had been. Apparently, Jordao and coworkers (30, 31, 32) and Johansson (28) are the only investigators who have tested the statistical significance of their observed differences in gestation length of dams at various ages. Of these investigators, Johansson, who found that the first gestations averaged 1.1 days less than the average of all gestations, was the only one to find that the difference was significant. Likewise, the duration of gestation in goats and sheep is reported to increase slightly until they reach 6 and 8 yr. of age, respectively (3, 4).

TABLE 6

Mean gestation length for dams of different ages

Age of dams	No. of gestations	Mean length
(*yr.*)		(*d.*)
2 or less	255	277.28
3	219	278.25
4	150	278.81
5	97	279.28
6	68	278.60
7	43	279.26
8	38	278.26
9 or more	64	278.00
5 or more	310	278.74
Total gestations	934	278.24

RESULTS AND DISCUSSION

The data used were restricted to the Ayrshire, Holstein-Friesian and Jersey gestations used in the first part of this study. These three breeds were used because the differences between their mean gestation lengths were not significant. The 2-yr.-old dams averaged 277.28 days and the mean of the dams 5 yr. old and over was 278.74 days (table 6). The average gestation length appears to increase slightly until the dams reach 5 yr. of age. The difference of 1.46 days between the mean gestation length at 2 yr. of age and 5 yr. and over is highly significant, giving a t-value of 3.35 with 563 degrees of freedom. These results substantiate those of Johansson (28). When considering the difference in gestation length between 2-yr.-olds and mature cows, it is pertinent to note that Ineichen (26) found among cows of the same age that the gestation length of heavier cows exceed that of lighter cows by 2.9 days. It was not possible to study the effect of this factor on these data because the weights of the cows were lacking.

E. SEX OF THE FETUS AS A FACTOR AFFECTING GESTATION LENGTH

The majority of the studies concerning the effect of the sex of the fetus on gestation length have shown that the male is carried about 1 day longer than the female (2, 5, 6, 8, 10, 17, 23, 24, 26, 28, 30, 31, 34, 35, 36, 42, 46, 47, 48, 52, 54). In three cases (28, 40, 54) the time that the male fetus spent *in utero* was reported to be significantly longer. In other investigations (34, 45, 55, 57, 59) it was concluded that the mean duration of pregnancy was not affected by the sex of the fetus.

RESULTS AND DISCUSSION

These data included the same 1,256 gestations used in the first part of this study. The mean gestation length preceding the birth of both male and female calves is shown separately in table 7 for each of the five breeds and for the five breeds combined. Gestations preceding the birth of male calves averaged 0.77 days longer than gestations preceding the birth of female calves. The significance of the differences of the means of the gestation lengths was tested by calculating the *t*-values (50). Mean differences in both the Guernsey and Holstein-Friesian breeds were significant at the 1 per cent level and the mean difference of all breeds combined was significant at the 2 per cent level. Mean differences of the other breeds, including the Brown Swiss, in which the gestations preceding birth of females exceeded that for males by 0.73 days, could have been due to chance alone. This offers a logical explanation of the contradictory results of other studies; since the mean difference is small, it may be misleading when samples are relatively small.

TABLE 7

Mean gestation length preceding birth of male and female calves

Breed	Male		Female		*t*-value	$t_{0.05}$
	(*no.*)	(*d.*)	(*no.*)	(*d.*)		
Guernsey	131	283.44	146	281.97	2.63**	1.97
Holstein-Friesian	191	279.25	163	277.77	2.62**	1.97
Jersey	117	278.30	132	277.49	1.07	1.97
Ayrshire	172	278.31	159	278.01	0.64	1.97
Brown Swiss	20	287.95	25	288.68	0.59	2.02
All 5 breeds	631	279.96	625	279.19	2.44*	1.96

* $P > 0.01 < 0.02$
** $P < 0.01$

F. CORRELATION BETWEEN GESTATION LENGTH AND BIRTH WEIGHT OF THE CALF

Hammond (21) reported that within a number of species an association had been found between birth weight and viability. He explained that some of the essential physiological functions develop late in fetal life so that birth weight serves as an indication of the stage of development. In dairy cattle, due to interspecific differences in stage of development at birth, further clarification of these processes is necessary before the importance of birth weight in relation to viability can be established definitely.

The rate of gain of beef calves is influenced by birth weight (1). Correlation coefficients ranging from + 0.39 to + 0.68 have been found between birth weight and body weight of beef cattle at various ages (10, 37, 38). In beef cattle, highly significant correlations have been found by three investigators (34, 37, 42) between length of gestation and birth weight. Dawson *et al.* (10) found a low but significant correlation between these two factors and Paim (48) found no correlation. In the case of dairy cattle, there is less evidence to indicate a correlation between these factors. Bonadonna and Valerani (6) reported a correlation of + 0.30 between gestation length and birth weight in Brown Alpine and Friesian cattle. Studies made by others (12, 14, 17, 45) have shown little or no correlation between gestation length and birth weight in dairy cattle. The correlation between birth weight and the length of pregnancy in humans has been found to range from + 0.40 to + 0.50 (33).

RESULTS AND DISCUSSION

In table 8 the number of calves, mean birth weight, and correlation coefficient are shown for each breed, based upon all the gestations used in the earlier parts of this study on which birth weights were available. The only significant correlation coefficients are in the Ayrshire, Brown Swiss and Jersey breeds. Therefore, it appears likely that a smaller correlation exists between gestation length and birth weight in dairy cattle than in beef cattle. These very limited data indicate that, within the dairy breeds, a correlation between gestation length and birth weight may be restricted to those breeds in which the calves show more condition at birth.

TABLE 8

Relation between gestation length and birth weight

Breed	No. calves	Mean gestation length	Mean birth weight	Correlation coefficient	Probability
		(*d.*)	(*lb.*)		
Ayrshire	96	278.70	79.07	+ 0.26	< 2%
Brown Swiss	20	289.05	100.85	+ 0.54	< 2%
Guernsey	78	283.14	67.55	+ 0.15	< 20%
Holstein-Friesian	64	277.31	90.59	+ 0.24	< 10%
Jersey	63	276.79	52.38	+ 0.29	< 5%

G. SEASONAL EFFECT ON GESTATION LENGTH

In most cases, the studies concerning the seasonal difference in gestation length indicate that winter freshening cows have the longest gestations and summer freshening cows have the shortest (2, 23, 28, 44, 46, 47). The mean differences reported by these investigators range from 0.8 to 3 days. Ineichen (26), whose study included 3,105 gestations, found that the gestations terminated by spring births were longest and that the shortest gestations preceded the autumn births. It was possible to determine the significance of these differences only in Johansson's study (28) in which the difference of 2.4 days between the months of February and August was greater than twice the standard error of the difference.

As can be expected when the differences are small, others (12, 27, 34, 45) found no difference or no significant difference in the mean duration of pregnancy of cows freshening at different seasons of the year. Blum (5) and Ineichen (26) attribute the seasonal increase in gestation length to the difference in environmental influences, such as lack of green feed, sunlight and exercise during the winter season, and to the seasonal difference in the frequency of using the sires. A reduction of 2.3 days in length of pregnancy preceding autumn and winter births compared to spring and summer births also has been reported for humans (3).

RESULTS AND DISCUSSION

The Ayrshire, Holstein-Friesian and Jersey gestations grouped according to the month of calving (table 9) show a definite trend, with the exception of the months of February and March. The greatest difference between months, 3.29

TABLE 9

Duration of pregnancy according to the month and season of calving

Mo.	No. of gestations	Av. length	Season	Av. length
		(*d.*)		(*d.*)
December	91	278.66		
January	87	279.40	Winter	278.59
February	83	277.67		
March	69	278.22		
April	88	279.88	Spring	279.02
May	94	278.80		
June	71	278.93		
July	61	278.05	Summer	278.17
August	78	277.56		
September	52	277.65		
October	72	276.89	Autumn	276.95
November	88	276.59		
Av.		278.24		

days, occurs between April calvings, which are the longest, and those of November which are the shortest. The analysis of variance shows the mean difference between months to be highly significant with an F-value of 3.09. The difference of 2.07 days between the spring (March, April and May) and autumn (Sept., Oct. and Nov.) seasons is highly significant, a t-value of 4.38 with 461 degrees of freedom being obtained. Compared to previous studies, these results are more similar to those of Ineichen (26) than to the observations of other investigators. Considerable variability apparently exists between different herds in the factors which are responsible for seasonal variation in gestation length.

SUMMARY AND CONCLUSIONS

The relationship between certain factors and the duration of pregnancy was investigated in a purebred dairy herd composed of the five major breeds of dairy cattle. Data used were restricted to gestations terminated by the birth of a living, single normal-appearing calf. Although not included in the data used in

this study, the gestations preceding the birth of twins, in which at least one of each pair was alive at birth, averaged 3.4 days less than gestations terminated by single births.

The means of the Brown Swiss and Guernsey gestations were 288.4 and 282.7 days, respectively. The mean duration of pregnancy for each of these two breeds was different from that of each other and from that of each of the other three breeds at the 1 per cent level. The means for Ayrshires, Holstein-Friesians and Jerseys, which were 278.2, 278.6 and 277.9 days, respectively, were not significantly different from each other. The variances of the mean gestation length were significantly less in the two breeds where more line breeding had been practiced.

Upon comparing the mean gestation lengths resulting from the use of different sires within four of these breeds, inter-sire difference was significant in three breeds but not in the Ayrshire breed in which the sires were more closely related.

Correlations of +0.32 and +0.20, significant at the 1 and 5 per cent levels, respectively, were found between the time the dam spent *in utero* and the time she carried her progeny. Apparently this is the first time that correlation coefficients have been reported between the duration of pregnancy of a dam and of her progeny. The difference of 1.5 days by which the mean gestations of cows 5 yr. old and over exceeded that of 2-yr.-olds was highly significant.

Gestations preceding the birth of 631 male calves averaged 0.77 days longer than 625 gestations preceding the birth of female calves. Although this difference was small, it was found to be significant. The correlation between gestation length and birth weight was significant in the Ayrshire, Brown Swiss and Jersey breeds but not in the other two breeds. The gestations of spring-calving cows averaged 2.07 days longer than fall-calving cows. This difference was significant at the 1 per cent level.

The following evidence indicates genetic influence on gestation length: (a) differences between breed means, (b) smaller variances within the breeds in which line breeding was practiced, (c) inter-sire differences within breeds, (d) decrease in inter-sire differences when sires are closely related, and (e) positive correlation between time the dam and her progeny spent *in utero*.

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FROZEN HOMOGENIZED MILK. VIII. EFFECT OF THE ADDITION OF SUCROSE AND ASCORBIC ACID ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

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Previous studies (1, 2, 3, 4, 5, 6, 7) have revealed that various factors affect the keeping quality of frozen homogenized milk. A more recent study (8) has shown that samples of commercial chocolate drinks did not deteriorate in flavor or separate to an objectionable degree when thawed after 380 days of storage at −17.8° C. Since sucrose is an important ingredient of chocolate drink, it could be possible that sucrose was a major factor contributing to the long storage life of frozen chocolate drink. The addition of ascorbic acid has been reported to prolong the time that frozen homogenized milk can be held (6).

These experiments were undertaken to determine if the addition of sucrose or sucrose plus ascorbic acid to homogenized milk would further increase the time it could be held frozen without objectionable quality deterioration.

EXPERIMENTAL RESULTS

Homogenized milk with a fat content of 3.8 per cent, pasteurized by holding at 68.3° C. for 30 min. and packaged in 0.5-pt. paper containers by a commercial dairy was used.

To determine if the addition of sucrose to homogenized milk would increase the time the milk could be held frozen, the samples were divided into four sets and a 50 per cent sucrose solution was added in such quantities that sets 1, 2, 3 and 4 contained 0.5, 1.0, 1.5 and 2.0 parts of sucrose per 100 ml. of milk, respectively. No sucrose solution was added to a fifth set which served as control samples. The upper limit in quantity of sucrose added was established by determining the point above which the sucrose-milk mixture had an objectionable sweetness. After the addition of the sucrose solution, the samples were mixed, frozen and stored in the original containers at −23.3° C.

Samples were removed from storage at periodic intervals, thawed overnight at refrigerator temperature (4 to 6° C.), and examined for flavor and separation. The degree of separation was measured by determining the sedimentation of 50 ml. by centrifuging, as previously reported (1, 6). Flavor determinations were made by experienced milk judges. Table 1 reveals that sucrose retarded the separation of frozen homogenized milk when added in quantities ranging from 0.5 to 2.0 parts per 100 ml. of milk. Separation was not noticeable in the milk samples containing sucrose until they had been stored 185 days. Notice-

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able separation had taken place in the control sample after a storage of 100 days.

It was previously reported (1) that when frozen homogenized milk was held for 89 days at a constant temperature of – 10° C. a sedimentation of 1.5 ml. occurred. Control samples in the present experiment which were frozen and stored at a temperature of – 23.3° C. showed a noticeable separation, as indicated by 0.8 ml. of sedimentation after 100 days. The addition of sucrose delayed this noticeable separation or flocculation for 85 days and the milk remained normal for 185 days of storage. Table 1 shows that the addition of sucrose increased the time the frozen homogenized milk remained normal in appearance when thawed.

To determine if ascorbic acid and sucrose would be more effective than sucrose alone, a second series of samples was prepared. The homogenized milk used was of the same quality and was packaged in the same manner as that used in the first series. The samples were divided into seven groups.

TABLE 1

Influence of sucrose on the stability of frozen homogenized milk

Sample	Quantity of sucrose Parts/100 ml. milk	No. of days frozen (frozen and stored at –23.3° C.)												
		6	36	63	75	91	100	109	128	144	165	185	199	234
(*no.*)		*Degree of sedimentation (ml.)*												
1	0.5	0.02	0.02	0.02	0.06	0.05	0.07	0.05	0.04	0.05	0.07	3.0	9.0	8.0
2	1.0	0.02	0.02	0.02	0.05	0.05	0.05	0.02	0.04	0.10	0.05	1.6	5.0	2.5
3	1.5	0.02	0.02	0.02	0.08	0.05	0.05	0.05	0.05	0.06	0.04	0.5	1.2	0.80
4	2.0	0.02	0.02	0.02	0.03	0.06	0.05	0.02	0.04	0.07	0.04	0.35	0.55	0.15
5	0.0	0.02	0.02	0.06	0.10	0.14	0.8	0.48	0.2	0.70	1.8	11.0	13.0	13.0

Ascorbic acid, 100 mg. per liter, was added to groups 1, 2 and 3. Quantities of a 50 per cent sucrose solution were added to groups 1, 2 and 3 and to groups 4, 5 and 6 so that each of these groups contained 1.0, 1.5 and 2.0 parts, respectively, of sucrose per 100 ml. of milk. The seventh group constituted the control samples. The samples were frozen in the original containers at – 23.3° C. and stored at – 17.8° C. The storage at a higher temperature provided a more severe test of the stabilizing effect of the sucrose and the sucrose with ascorbic acid. As previously shown (1), the higher the storage temperature of frozen homogenized milk the more rapid the deterioration in its appearance and flavor. The same procedures of thawing, separation determination and flavor determination were followed as in the first series.

Table 2 shows that after 78 days excessive separation had occurred in the control sample. Separation was slightly noticeable in samples 1, 5 and 6 when thawed after 112 days of storage. After 129 days of storage, separation was found in all the samples. Samples 4 and 5, which contained only sucrose, showed more separation than samples 2 and 3, containing sucrose with ascorbic acid. After 145 days of storage, separation was not excessive in the samples containing

sucrose with ascorbic acid, especially in those samples containing 1.5 and 2.0 parts sucrose per 100 ml. of milk.

In the first series of samples (table 1) in which varying quantities of sucrose were added, the control sample was found to be slightly oxidized after 36 days of storage and definitely oxidized after 75 days. Samples 1 and 2 were slightly oxidized after 75 days and samples 1, 2 and 3 were definitely oxidized after 91 days. Sample 4 was slightly oxidized after 91 days and definitely oxidized after 100 days .

In the second series of samples, in which varying quantities of sucrose and varying quantities of sucrose with ascorbic acid were added, the control sample was slightly oxidized when thawed after 78 days, and definitely oxidized when thawed 129 days after freezing. Samples 4, 5 and 6, which contained only sucrose, were slightly oxidized when thawed after 112 days and definitely oxidized when thawed after 145 days. Samples 1, 2 and 3, which contained ascorbic acid, were free of oxidized flavor when thawed after 212 days of storage. These results are

TABLE 2

Influence of sucrose with ascorbic acid on the stability of frozen homogenized milk

Sample	Sucrose	Ascorbic acid	No. of days frozen (frozen at −23.3° C.: stored at −17.8° C.)						
			6	78	112	129	145	177	212
(*no.*)	(*Parts/ 100 ml.*)	(*Mg./ 100 ml.*)	*Degree of sedimentation (ml.)*						
1	1.0	10	0.03	0.04	0.5	2.2	4.2	6.5	13.0
2	1.5	10	0.03	0.10	0.12	0.75	1.2	3.5	10.5
3	2.0	10	0.03	0.15	0.12	0.50	0.9	1.6	8.0
4	1.0	0	0.03	0.32	0.2	2.2	10.5	14.0	15.0
5	1.5	0	0.03	1.0	0.5	3.5	8.0	11.0	14.0
6	2.0	0	0.03	0.38	1.0	3.5	9.5	11.0	13.0
7	0	0	0.03	11.5	11.0	14.5	15.0	15.0	17.0

similar to those previously obtained by adding sodium citrate and ascorbic acid (6). However, in several instances, the samples containing two parts of sucrose per 100 ml. of milk were criticized as being sweet. This sweetness probably would not have been noticeable if milk with lesser degrees of sweetness had not been available for comparison.

SUMMARY AND CONCLUSIONS

Sucrose in concentrations of 0.5, 1.0, 1.5 and 2.0 parts per 100 ml. of homogenized milk frozen and stored at −23.3° C. retarded for 185 days a noticeable separation in the thawed milk. Samples with 0.5, 1.0 and 1.5 parts of sucrose were definitely oxidized when thawed after 91 days of storage. Samples with two parts of sucrose were definitely oxidized when thawed after 100 days of storage. The control sample showed noticeable separation when thawed after 100 days and was definitely oxidized when thawed after 75 days of storage.

A second series of samples containing sucrose in concentrations of 1.0, 1.5 and 2.0 parts per 100 ml. of homogenized milk frozen at −23.3° C. and stored at −17.8° C. did not show definitely noticeable separation when thawed until 129

days of storage. The samples were slightly oxidized when thawed after 112 days and definitely oxidized when thawed after 212 days of storage. Additional samples with 1.0, 1.5 and 2.0 parts sucrose plus 10 mg. of ascorbic acid per 100 ml. of milk showed noticeable separation when thawed after 129 days of storage, but were free of oxidized flavors when thawed after 212 days of storage. The control sample for this series showed excessive noticeable separation and was found to be slightly oxidized when thawed after 78 days of storage. At 129 days, the control sample was definitely oxidized.

Sucrose increased the time frozen homogenized milk remained normal in appearance when thawed and also slightly retarded the development of an oxidized flavor. The addition of sucrose with ascorbic acid to homogenized milk, frozen at $-23.3°$ C. and stored at $-17.8°$ C., increased the time the milk remained normal in appearance and more than doubled the time it remained normal in flavor.

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GENETIC CORRELATION BETWEEN TYPE AND PRODUCTION IN JERSEY CATTLE[1]

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Breeders of dairy cattle have long been searching for some easily measured characteristic which would indicate breeding and producing ability for butterfat production. Since the over-all type or conformation of an animal is given much attention in the show ring, the public sales and more recently in herd classification programs, it is natural to inquire whether type is correlated closely enough with production to serve as such an indicator.

A phenotypic correlation between two characters in the same animal can result from two entirely different kinds of causes. First, one or more of the variations in the environment which happened to that individual may have affected both the characters. Second, one or more of the genes may have affected both characters alike or oppositely. It follows that selection for one of two characters which are positively correlated in the individual need not improve the other character in the next generation. In fact, the opposite will result if the genetic part of the correlation is negative but the environmentally caused portion is positive and larger.

The path coefficient diagram in figure 1 shows in its simplest form this dual nature of a phenotypic correlation. The observed correlation (r_{TP}) is made up of a genetic component, $g_T x g_P$, and an environmental component $u_T w u_P$. T and P are the observed type and production. G_T and G_P indicate the genic[4] values of the individual for type and for production, respectively. U_T and U_P indicate all other causes of individual differences in T and in P.

A correlation (x) between G_T and G_P might be caused by a variety of circumstances, notable among which are (a) pleiotropic effects of single genes, (b) linkage between genes affecting T and genes affecting P (which is a cause only when the coupling double heterozygotes are more abundant than the repulsion

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[3] The American Jersey Cattle Club permitted the use of its data and also helped meet the costs of the computations.

[4] Genic, as used in this article, is synonymous with "additively genetic" and "general breeding" values. It is the sum of the average effects of all genes in the individual. Any differences between the average effects of a gene and special effects which it may have in some combinations but not in others (the "dominance deviations" and "epistatic deviations") are put by the method of analysis mostly into U_T and U_P. Therefore, if dominance and epistasis are important, the U_T and U_P actually include a bit more than the purely environmental effects. However, for brevity and because for practical purposes under the usual systems of mating the dominance and epistatic deviations act nearly the same as environmentally caused deviations, we will refer to G and U as if they meant heredity and environment, respectively.

ones or vice versa), and (c) heterogeneity in the previous breeding practices in the population. Examples of the latter are that if some breeders had striven only to improve type and others only to improve production, and if both groups had had some success, this would tend to make a negative x, while if all had striven toward the same goal but with unequal success this would have tended to make a positive x, etc.

The accuracy with which a breeder can use a cow's type to predict her breeding value for fat production depends wholly on r_{TGP}, which equals xg_T. On the other hand, the accuracy of predicting a cow's own producing ability from her type is r_{TP} itself, which can be larger or smaller than r_{TGP} or even opposite to it in sign.

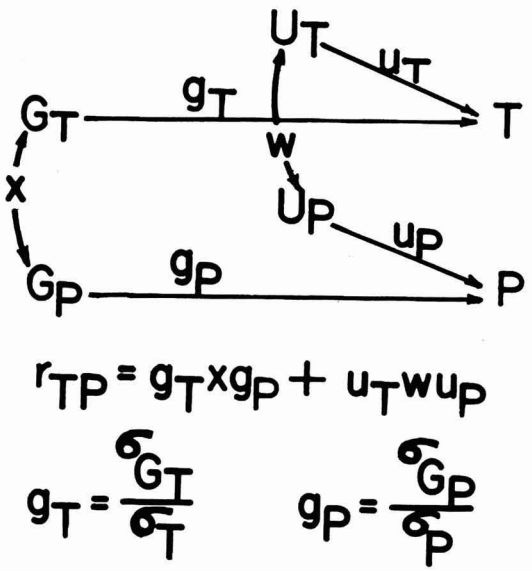

FIG. 1. Path coefficient diagram showing the biometric relations between type and production of the same animal.

The primary objectives of the present study were to obtain reliable estimates of the heritability of type (g_T^2) and production (g_P^2) and of the genetic correlation (x) between type and production.

DATA USED

From the American Jersey Cattle Club were obtained all the data on type and fat production in 245 herds which were on Herd Improvement Registry test for at least 4 of the 5 yr. from 1943 to 1947, inclusive. Although 39 states were represented, most of the herds were located in the northeastern and midwestern states. Altogether there were 8,464 cows which each had an official type rating and one to five production records. The average number of production records per cow was 2.01.

All records of fat production were adjusted to a twice-a-day milking, mature-equivalent basis by the American Jersey Cattle Club. If the actual record was for more than 305 days, only the first 305 days were used. If the record was for less than 305 days, but was recorded as a complete lactation for that cow, no adjustment for length was made. No record of less than 270 days duration was used in the present study. The increased variation caused by using the few records that were shorter than 305 days was less than 0.5 per cent of the intra-herd variance.

The date of type classification or the name of the inspector doing the classifying were not noted, but doubtless many of the cows were classified for type before 1943. The rules of the classification plan provide that, if a breeder enrolls in the program, all eligible animals then in his herd must be classified. He cannot offer for classification only a select few. If a cow is classified more than once, only the highest rating she has received is used. Which of the cows in these data had had their type rating raised on reclassification was not noted.

Under the rules of the classification program a cow is placed into one of six grades: Excellent, very good, good plus, good, fair or poor. When a cow is classified poor, her registration is cancelled at once. Hence no poor cows appeared in the data studied here. For the analysis the grades were given consecutive numerical scores beginning with 2 for a fair cow through 6 for an excellent cow.

METHOD USED TO ELIMINATE THE YEAR EFFECTS

Daughters and dams will have most of their records in different years. Therefore, any effects of environmental changes from one year to another within the herd would bias the regression of daughter on dam downward if such effects are not eliminated. This made it seem necessary that the effects of differences between years within each herd be removed. The importance of doing this depends, of course, on whether the effects of year-to-year variations within herd really are large. Solving this problem by expressing each record as a deviation from the yearly herd average and subsequently working only with such deviations was tried but was not satisfactory. Since not all the cows had a record in every year, the differences between years, which would thus be removed, actually would contain some differences between cows. Those differences which would remain between records made in different years by the same cow would still contain some cow differences which could not be separated easily.

The linear mathematical model for the analysis given in table 1 is:

$$P_{ijk} = \mu + h_i + y_{ij} + c_{ik} + e_{ijk}$$

where P_{ijk} is the production of the k^{th} cow in the j^{th} year in the i^{th} herd: μ is the mean of all records, h_i is the effect of the i^{th} herd, y_{ij} is the effect of the j^{th} year in the i^{th} herd, c_{ik} is the effect of the k^{th} cow in the i^{th} herd, and e_{ijk} is the error associated with the P_{ijk}. Estimates of the variance components for year, cow and error (Y, C and E) are obtained by setting the expectations of the mean squares in table 1 equal to the computed values and solving the resulting set of equations. When the analysis is on an intra-herd basis, h_i disappears from the model.

TABLE 1

Analysis of variance used to obtain estimates of the error (E), cow (C) and year (Y) components

Source of variation	d.f.	Expectation of mean square
Within herd	$\sum_i (n_i-1)$	
Between years	$\sum_i (r_i-1)$	$E + \bar{c}_2C + \bar{y}_2Y$
Within years	$\sum_i (n_i-r_i)$	$E + C$
Within herd	$\sum_i (n_i-1)$	
Between cows	$\sum_i (k_i-1)$	$E + \bar{c}_1C + \bar{y}_1Y$
Within cows	$\sum_i (n_i-k_i)$	$E + Y$

Where: n_i = no. of records in the i^{th} herd.
r_i = no. of years in the i^{th} herd.
k_i = no. of cows in the i^{th} herd.

This analysis was done by Legates (2) on all records of these 8,464 cows and also of the 3,941 other cows which made records in herds on HIR test in the same period but were not classified for type. The estimate of Y was not made separately in the present study for the cows which had type ratings, but the number of year components in all mean squares for production among these cows was calculated. Since there appears to be no reason for the year effects to be different among those cows with type ratings and among those not classified for type, the estimate of Y obtained by Legates was used here for obtaining estimates of the other components.

DAUGHTER-DAM ANALYSIS

Number of pairs. There were 2,044 dams which had 2,786 daughters. The dam and her daughter(s) were included only when they made all their records in the same herd. These daughter-dam pairs were distributed among 226 of the 245 herds. The average number of production records was 2.46 per dam and 1.89 per daughter.

Means of the type ratings and fat production records of dams, daughters, and and all cows are given in table 2. The dams averaged less than their daughters in fat production but more than all cows, whereas the dams exceeded both the daughters and all cows in their type ratings.

TABLE 2

Means of type ratings and fat production records of dams, daughters and all cows

	No. of cows	Means	
		Production	Type
All cows	8,464	433	4.19
Dams	2,044	439	4.30
Daughters	2,786	445	4.17

TABLE 3

Analysis of variance and covariance of butterfat production of dams and daughters (Mean squares only)

Source of variation	d.f.	Mean sq. or Cov.[a]	Expectation of mean square or Covariance	Components
		P'		
Total	2043	9,189	$E_{P'} + 0.531\ Y + 0.992\ H_{P'}$	$Y = 515.5$[b]
Among herds	225	41,594	$E_{P'} + 2.717\ Y + 9.012\ H_{P'}$	$H_P = 3900$
Intra-herd	1818	5,178	$E_{P'} + 0.260\ Y$	$E_P = 5044$
		P P'		
Total	2784.3	4,812	$G_{P'}/2 + 0.204\ Y + 0.992\ H_{P'}$	$Y = 515.5$[b]
Among herds	351.4	33,869	$G_{P'}/2 + 1.532\ Y + 7.864\ H_{P'}$	$H_{P'} = 4129$
Intra-herd	2432.2	616	$G_{P'}/2 + 0.012\ Y$	$G_{P'}/2 = 609.7$

[a] A prime identifies the dam's performance.
[b] This estimate was obtained in a separate analysis as explained in the text.

For estimating heritability in dairy cattle data, the regression of daughter on dam generally is preferred to the correlation, since the dams are likely to be more highly selected. Heritability, the parameter to be estimated, is the fraction of the total or phenotypic variance in the dams that is transmissible to the progeny. The daughter of the dam is used merely as an indicator for the dam's transmitting ability. The dam's genic effects will, on the average, have only one-half as large an effect on her daughter's records as on her own, since a daughter receives a sample half of the genes which her dam had. Hence, there is the necessity of doubling the regression of daughter on dam when estimating what fraction of the phenotypic variance among dams was caused by differences among the dams in transmitting ability.

Use of an average of n records. When the average production of n records on the dam is used, (tables 3 and 4), the E component is different from the E component in individual records (table 1). In table 1 it is the effect of the temporary environmental variations on a random individual record, while in tables 3 and 4 it contains only $\frac{1}{n}$ of that but also includes all the effects of real differences between cows in the same herds; *i.e.*, it also includes C of table 1.

TABLE 4

Summary of components of variance and covariance

Component	Source of estimate			Genetic components	Estimate
	Dams	Daughters	All cows		
E_T	0.5495	0.5034	0.5430	$G_T/2$	0.0390
E_P	5044	4479	4961	$G_P/2$	609.7
H_T	0.1166	0.1069	0.1167	Cov $(G_T G_P)/2$	1.054[a]
H_P	3900	4464	3814	Cov $(G_T G_P)/2$	0.746[b]
Cov (TP)	8.162	7.096	7.419		
Cov_H (TP)	2.553	5.558	4.110		

[a] Estimated from the production of dam and type of daughter.
[b] Estimated from the type of dam and production of daughter.

Table 3 shows, as an example, how the variance and covariance were analyzed to estimate heritability of production. In the analysis made by Legates (2), in which these data were included, the Y component was estimated to be 515.5. This value for Y was used in all analyses here when estimating other components.

Table 3 shows no error component in the covariance. This is true if the intra-herd permanent and temporary environmental effects were not correlated as between daughter and dam. The inter-herd differences, both environmental and genetic, went into the H component. The dominance deviations and most of the epistatic deviations are not correlated between parent and offspring. The intra-herd environmental effects on dam and daughter would not be correlated unless dam and daughter were highly contemporary and the year effects had not been removed, or unless breeders treated daughter and dam alike but differently from other pairs in the same herd. General time trends could not be important here, since these data covered only the short space of 5 yr.

The fractional degrees of freedom in table 3 result from the fact that some of the dams had more than one daughter. The number of G/2 components which go out with the herd crossproduct is greater than the number of herds. The expected number of G/2 components in the various crossproducts is based simply on the biological fact that the expected resemblance between a dam and any one of her daughters is the same.

Heritability of production. The intra-herd regression of daughter's production on dam's production, presumably freed from year differences, as calculated from table 3, is $\frac{609.7}{5044} = 0.121$. From this the heritability of differences in fat production records made in the same herd and year, when adjusted to a single-record basis by the formula given by Lush and Straus (4), is estimated to be 0.18 ± 0.03. Ignoring the effects of years, the intra-herd regression of daughter on dam is 0.119, and heritability on a single-record basis is 0.17 ± 0.03. The repeatability figures used in adjusting these regressions to a single-record basis were 0.46 and 0.41, respectively. These are the estimates obtained by Legates (2) from all 12,405 cows.

Two primary reasons account for the heritability of fat production being only slightly higher when the effects of years were removed than when those effects were ignored. First, averaging all the records available on the dam diminishes considerably the amount of the year component remaining in the intra-herd variance of the dam. Second, the differences between years within herd accounted for only a small portion of the intra-herd variance; *i.e.*, Y in table 3 is small compared to E_P.

The year effects in the intra-herd covariance will always be small unless there is a deliberate effort to compare only the records which the dam and her daughter made in the same year. If the first record of the dam and of daughter are used there will be no year component at all in the covariance, except in those rare cases in which the daughter and the dam were both in milk the first year testing began in that herd. The small coefficient (0.012) of Y in table 3 shows the actual situation in these data.

On an intra-herd basis the dams were as variable in production as were all 8,464 cows (table 4), even though the dams presumably were a selected group. However, the selection for fat production seems not to have been very intense, since the dams averaged only about 5 lb. of butterfat more than all cows (table 2). Since this selection on fat production doubtless was combined with some selection for type, little decrease in the variance of the dams is to be expected. The reasons for the intra-herd variation in the daughter's production being significantly lower are not clear. E_P plus H_P equals almost exactly the same sum for daughters as it does for dams (table 4). But the daughters form more uniform groups within each herd (E_P for them is less) and differ more distinctly from herd to herd (H_P for them is larger). A part of this may be due to the closer intra-herd relationship of the daughters as compared to the dams. On the other hand, the dams had more records each than the daughters and this should have tended to make the dams less variable.

Heritability of type. The components of variance and covariance of type and production of daughters and dams are summarized in table 4. The intra-herd regression of type of daughter on type of dam is 0.071, and heritability is estimated to be 0.14 ± 0.04. This estimate is only half that given by Tyler and Hyatt (5) for Ayrshires from an intra-sire regression of daughter on dam (1,601 pairs) where all daughters and dams within sire were classified by the same judge on the same day. Most of the differences between judges are eliminated from the intra-herd regression given here, as most cows in a given herd were classified by one judge and on the same day. Since this was not always the case, some judge differences remain in both the numerator and denominator of the regression fraction.

In those herds classified more than once, some of the daughters and dams will have been classified by the same judge but in other pairs one judge will have classified the daughters and a different judge at a different time will have classified the dams. Thus, if judge differences exist, the variance of the dams (E_T) has been increased about 10 to 20 times as much as the covariance between dam and daughter ($G_T/2$). Consequently, to the extent that judges really differ in their ideal or in their mean rating levels, the estimate of heritability for type given here is expected to be lower than it would be if all type ratings had been made by the same judge. The difference between the intra-herd regression found here and the intra-sire and intra-judge regression reported by Tyler and Hyatt could possibly be some indication that judge differences are important. On the other hand, the heritability of differences in type might actually be lower in Jerseys than in Ayrshires. A third possibility, of course, is that the two values are equally valid and unbiased estimates of the same parameter, since the sampling errors are so large.

As seen in table 4, the dams were slightly more variable in type ratings than their daughters. The probability that so large a difference could be only a sampling error seems a little less than 0.01. One possible explanation for the difference is the regulation of the American Jersey Cattle Club which prohibits a cow from being classified "Excellent" until she has dropped her second calf.

Many more daughters than dams would not have had a second calf at the time of classification. Secondly, it seems likely that the judges were more conservative when classifying young cows, knowing that the rating they gave could never be lowered but might be raised on reclassification, according to the rules of the classification program.

Genetic correlation. This is estimated from the genetic variances and covariance of type and production as follows:

$$\frac{\text{Cov}(G_T G_P)/2}{\sqrt{(G_T/2)(G_P/2)}} = \frac{0.886}{4.875} = 0.181$$

The effects of differences between years in the same herd would be present in only $G_P/2$ if no attempt was made to remove such effects. By using averages, the year effects in this component are damped down so that they modify the estimate of the genetic correlation downward only a little, as compared with what it would be in a population where the general environmental effects did not change at all from 1 yr. to another. Since the expected value of either one of the variances or covariances is the same, regardless of the number of records per cow, the estimate of the genetic correlation is automatically on a single-record basis.

As pointed out by Hazel (1), the sampling error of a genetic correlation is necessarily very large, since it is calculated from four statistics which have some correlation between their sampling errors. A satisfactory method of obtaining fiducial limits on estimates of genetic correlations has not been devised.

Herd differences. Differences between herds accounted for about 18 per cent of the total variation in the type ratings of both the dams and daughters. Herd differences were considerably more important in production, where they accounted for 44 and 48 per cent of the total variations in the average fat production of dams and daughters, respectively. Although this gives some indication that production differences between herds are greater for daughters than for dams, it is doubtful whether this is statistically significant. Differences between herds can, of course, be caused by either environmental or genetic differences, but scattered bits of evidence and general considerations indicate that the environmental differences between herds generally are much larger than the genetic ones.

The regression of the herd mean fat production on the herd mean type classification score was 19.5 for dams and 41.5 for daughters. The correlation between herd means for type and production was 0.12 for dams and 0.23 for daughters. These differences between daughters and dams were not statistically significant.

APPLICATION OF THE RESULTS

Construction of indexes. By using the genetic and phenotypic constants obtained in this study and by following the principles of index construction, as given in detail by Hazel (1), two selection indexes were developed. The first was constructed by giving type one-third as much attention as production and the second, by giving both characters equal weight. By "one-third as much" is meant that an increase of one standard deviation in type would raise an animal's

index one-third as much as would an increase of one standard deviation in production.

No effort was made here to determine the relative economic importance of type and production. Undoubtedly, the relative economic importance is not actually the same in all herds of dairy cattle or even from time to time within the

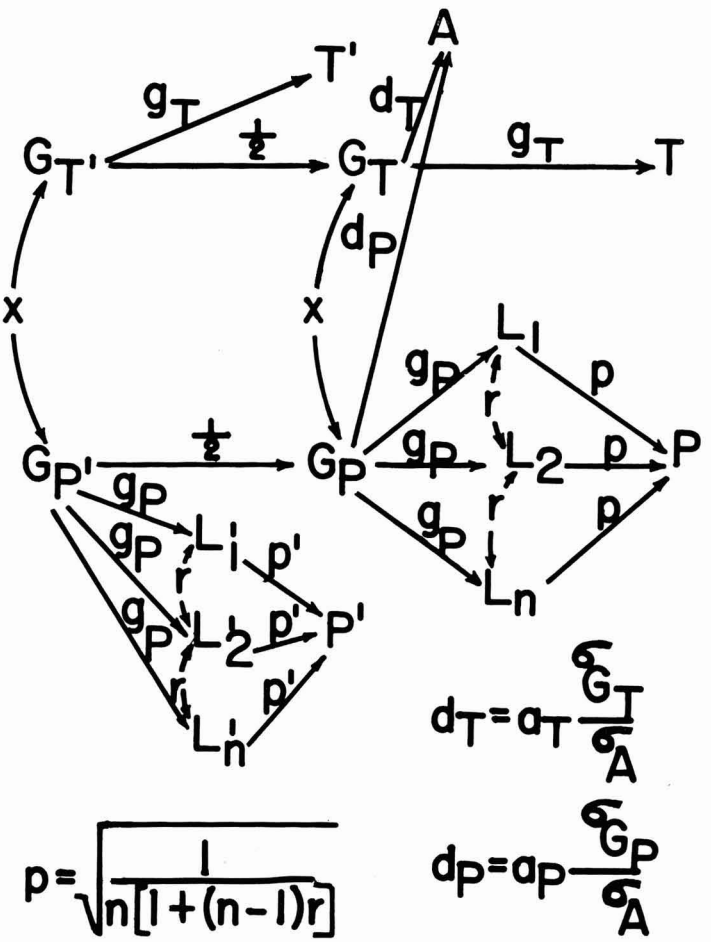

FIG. 2. Path coefficient diagram to illustrate the relations between the aggregate genotype for type and production (A) and the phenotypic measurements of these two traits on the cow being indexed and her dam.

same herd. It would vary with what the breeder's customers really want. Breeders who sell much of their stock through public sales or show ring advertising should pay more attention to type than a commercial dairyman whose income depends almost wholly on his milk or butterfat sales.

Only information on type and production of the individual being indexed and

of her dam were considered here in constructing the two indexes. The number of records of fat production were allowed to vary from none to four. Figure 2 gives the path coefficient diagram from which was derived the correlations between the phenotypic measurements (T', P', T and P) and the aggregate genotype, A, of the daughter which is being indexed. L' and L refer to the lactation record of the dam and daughter, respectively. The relative economic values of type and production are given by the a's. For constructing index no. 1 and 2 in these data, a_T was 39 and 116, respectively, whereas a_P was equal to unity in both indexes. The values for a_T are the assumed economic values (in pounds of butterfat required to equal a change of one grade in type) for the two situations considered. The values for the a's were determined from the intra-herd standard deviations of the two traits.

Value of R_{IA}. An index (I) is defined by $I = b_T\ T + b_P\ P + b_{T'}\ T' + b_{P'}\ P'$, where the b's are multiple regression coefficients which are calculated from the conventional set of four simultaneous equations. Calculating the b's in this manner makes the multiple correlation (R_{IA}) the maximum which these economic

TABLE 5

Constants used to construct the two indexes

Phenotypic constants	Genetic constants
$\sigma_P^2 = 7350$	$\sigma_{G_P}^2 = 1229$
$\sigma_P = 85.74$	$\sigma_{G_P} = 35.06$
$\sigma_T^2 = 0.54299$	$\sigma_{G_T}^2 = 0.07700$
$\sigma_T = 0.73688$	$\sigma_{G_T} = 0.27749$
$r_{TP} = 0.1174$	$g_P^2 = 0.1672$
$r_{TT'} = 0.0709$	$g_P = 0.4089$
$r_{PP'} = 0.0836$	$g_T^2 = 0.1418$
$r_{P'T} = 0.0139$	$g_T = 0.3766$
	$x = 0.1809$

values and an additive relation will permit. In other words, the estimate of the cow's breeding value for both type and production is made as accurate as is possible with the specified amount of information.

A convenient and practical method of eliminating yearly effects on the production records of individual cows has not been found. Since selections must be made with these yearly differences present, the genetic and phenotypic constants used in constructing the indexes were those estimated ignoring yearly differences. For convenience the necessary constants are assembled in table 5. σ_P^2 is given for single records of production. This was calculated by adjusting the intra-herd variance (5,171) from all 8,464 cows to a single-record basis. $\sigma_{G_P}^2$ was then calculated by assuming heritability to be 0.167 as estimated from the daughter-dam analysis when yearly differences were ignored. The genetic variance for type was calculated in a similar manner. The phenotypic correlations also are given as they would be with single records.

The b's and R_{IA}'s for the two indexes for selecting a cow when the number of records for her and her dam vary are given in table 6. In all cases where one or more records of production are available, it is assumed here that a type rating

TABLE 6

Partial regression coefficients for type and average production of the dam and daughter for different numbers of records of production and the multiple correlation between the index and the aggregate genotype for each combination, in two indexes for selecting among daughters[a]

No. records		b_T	b_P	$b_{T.}$	$b_{P.}$	R_{IA}
Dau.	Dam					
Index no. 1						
1	0	6.42	0.170	.....	.....	0.412
2	0	5.43	0.243	.....	.....	0.479
3	0	4.88	0.283	.....	.....	0.513
4	0	4.53	0.308	.....	.....	0.534
0	1	.....	.....	3.21	0.085	0.206
0	2	.....	.....	2.72	0.121	0.240
0	3	.....	.....	2.44	0.141	0.257
0	4	.....	.....	2.27	0.154	0.267
1	1	6.19	0.164	2.70	0.071	0.446
1	2	6.21	0.161	2.28	0.102	0.458
1	3	6.22	0.160	2.05	0.119	0.464
1	4	6.23	0.159	1.90	0.130	0.468
2	1	5.24	0.235	2.73	0.065	0.505
2	2	5.26	0.231	2.35	0.094	0.514
2	3	5.28	0.229	2.13	0.109	0.519
2	4	5.29	0.228	2.00	0.119	0.522
Index no. 2						
1	0	17.32	0.178	.....	.....	0.405
2	0	16.28	0.253	.....	.....	0.448
3	0	15.71	0.295	.....	.....	0.470
4	0	15.34	0.322	.....	.....	0.483
0	1	.....	.....	8.66	0.089	0.203
0	2	.....	.....	8.14	0.127	0.224
0	3	.....	.....	7.85	0.148	0.235
0	4	.....	.....	7.67	0.161	0.242
1	1	16.76	0.171	7.40	0.074	0.439
1	2	16.78	0.168	6.96	0.106	0.446
1	3	16.79	0.167	6.72	0.123	0.450
1	4	16.79	0.166	6.58	0.135	0.453
2	1	15.76	0.245	7.43	0.068	0.476
2	2	15.79	0.241	7.03	0.097	0.482
2	3	15.80	0.240	6.82	0.113	0.485
2	4	15.82	0.238	6.68	0.124	0.487

[a] Index no. 1 gives type one-third as much attention as production, whereas index no. 2 gives type and production equal attention. In all cases it is supposed that the cow has a type rating also if she has a production record.

also is known. When the amount of information on type and production is the same, R_{IA} is always higher in the first index than in the second. This is to be expected, since production is estimated to be slightly more heritable than type.

The amount of gain to be expected from selection, in terms of the aggregate genotype, varies directly with changes in R_{IA}, since

$$A - \bar{A} = R_{IA}\frac{\sigma_A}{\sigma_I}(I - \bar{I}).$$

For most conditions I should be almost normally distributed for any particular combination of information, so that if selection is by truncation,[5]

[5] Selection of truncation means merely that all individuals above a certain level of merit are retained for breeding and all those below that level are discarded.

$$A - \bar{A} = R_{IA}\ \sigma_A \frac{z}{b}$$

where z is the height of the ordinate at the point of truncation and b is the fraction saved for breeding.

Importance of increasing n. The last column of table 6 shows what is gained from increasing the number of records. The big gains from making n large come only when there is no information on the dam or when there is information on the dam but not on the daughter. These gains in accuracy are just half as much in the case of the dam as they are for the daughter. Likewise, R_{IA} is just half as large where there is information on the dam but none on the daughter. Where at least one record is available on the daughter, the gain from increasing n on the dam is relatively less than if one had nothing on the daughter at all.

Practical use of indexes presented. Unless the same amount of information is present on all cows being indexed, the type ratings and production records must be expressed as a deviation from some mean such as the herd average. In small herds, it probably would be best to express the production records as a deviation from the herd's moving average based on 3 or 4 consecutive yr., since the year-to-year differences in the average of a herd usually would contain more cow differences than in a large herd. For convenience, a round number close to the actual herd average may be used and changed only at intervals of 5 to 10 yr. Unless the actual herd average is changing rapidly, that will introduce little error.

An increase in R_{IA} could be made by utilizing available information on type and production from still other relatives. Since most selections of females are made either before freshening or shortly following their first or second lactations, little is lost by not considering the performance of their daughters. Paternal and maternal half sisters will increase the accuracy of the index materially if they are numerous and if little information is available on the cow herself or her dam. Full sisters are scarce and can be included most conveniently by counting each twice, once as a maternal sister and again as a paternal sister.

Naturally, the breeder likes to utilize for every cow all the information that makes her index more accurate. In order to do this with maximum accuracy, the b's must be determined separately for each possible combination of number of relatives and records and different kinds of relatives. Relatives which are less than 25 per cent related to the cow being indexed would rarely raise the accuracy enough to be worth considering. At least this would be true when she has records herself and there are records on her dam and some half sisters.

These practical difficulties in constructing and using an index, that would utilize all the information from those relatives which are 25 per cent or more related to the cow being indexed, can be overcome partially by some compromise. Since in most cases the b's vary only a little from one combination of information and relatives to another, one could use average values for the regression coefficients for type and production. However, maximum gain can be approached more nearly by another compromise in which the phenotypic measurement of each individual would be her most probable producing ability expressed as a deviation from the herd average. Then the b's for production would be those

applying only to the combinations of kinds and number of relatives with no record or with only one. The most probable producing ability has been defined previously by Lush (3) to be

$$\text{Herd average} - \frac{nr}{1+(n-1)\,r}\,[\text{Cow's own average} - \text{Herd average}]$$

where n is the number of records and r is the repeatability of records on the same cow. This step is unnecessary when comparing cows which all have the same number (n) of records, but either it or the use of different equations, as shown in table 6, are necessary for comparing fairly cows differing in n.

Using this relation to reduce actual average and n to "most probable producing ability" would do away with most of the variation in the regressions on production within each set of four lines in table 6. Then, since the regressions on type within each set of four lines do not vary much, one could use a convenient round number near the average of those and thus reduce the 32 lines of table 6 to the eight lines shown in table 7. The loss in accuracy would be small. Then,

TABLE 7

Average partial regression coefficients for type and most probable producing ability of the dam and daughter in two indexes for selecting among daughters[a]

No. records		b_T	b_P	$b_{T'}$	$b_{P'}$
Dau.	Dam				
Index no. 1					
1–4	0	5.3	0.42	.....	.....
0	1–4	.....	.....	2.7	0.21
1	1–4	6.2	0.40	2.2	0.18
2	1–4	5.3	0.40	2.3	0.16
Index no. 2					
1–4	0	16.2	0.44	.....	.....
0	1–4	.....	.....	8.1	0.22
1	1–4	16.8	0.42	6.9	0.19
2	1–4	15.8	0.43	7.0	0.17

[a] See footnote under table 6.

since the variation within the four lines for each index in table 7 is small, one could compromise by using an average for each column there and thus reduce index no. 1 to one equation and index no. 2 to another. The approximations would cost only a little in accuracy and would gain so much in simplicity that they seem feasible, even for field use. For example, index no. 1 might reduce to $5.6\ T + 0.41\ P + 2.4\ T' + 0.18\ P'$, while index no. 2 could be used as $16.3\ T + 0.43\ P + 7.3\ T' + 0.19\ P'$ with only small losses in accuracy.

For practical use one might want to divide the index through by one of the partial regressions in order to eliminate one multiplication when calculating an index value. For example, dividing the two reduced indexes given above by the respective partial regression for the most probable producing ability of the daughter and rounding would further simplify them as follows:

$$\text{Index no. 1} : 14\ T + P + 6\ T' + 0.4\ P'$$
$$\text{Index no. 2} : 38\ T + P + 17\ T' + 0.4\ P'$$

Selection for type as contrasted to selection for both type and production. Breeders of dairy cattle have often maintained that selection for type would also bring about improvement in production; in general, there seems to be some truth in this. However, if selection is based solely on the type of the cow, the genetic improvement in production will be only about one-sixth as fast ($r_{TG_P} = 0.068$ and $r_{PG_P} = 0.409$) as selection based solely on one production record of the cow in question. If type has no economic value at all, the genetic gain in production would be only about 0.5 per cent faster by considering type as well as production. A type rating on the cow being indexed and on her dam is about 18 per cent as valuable in predicting her breeding value for production as one of her own production records, or 36 per cent as valuable as one production record of her dam.

Many breeders cull heifers before they have made a production record. This culling often is done on the basis of the heifer's own type and both type and production records on the dam. Where type is one-third as valuable as production, such a system of selection will yield genetic progress about 60 to 65 per cent as fast as a system where the heifers are allowed to complete their first lactation prior to culling. If type is economically as valuable as production, the genetic gain would be about 75 to 80 per cent of what it would be by waiting for a production record on the daughter. R_{IA} in the first case varies from 0.26 to 0.31 and in the latter case from 0.34 to 0.36, depending on the number of records on the dam.

SUMMARY

The correlation between transmitting ability for type and transmitting ability for production was estimated as 0.18 from 2,786 daughter-dam pairs in the Jersey breed. The data came from 226 herds which had been on HIR test for at least 4 of the 5 yr. 1943 to 1947, inclusive. Additively genetic variation constituted about 18 per cent of the intra-herd and intra-year variance in single records of fat production and 14 per cent of the intra-herd differences in official type ratings.

Selection on the basis of type alone should therefore automatically bring about some genetic improvement in production. However, selection on type alone would require about 6 to 10 generations to obtain the improvement that selection on the basis of production would obtain in only one generation. Postponing selection until the daughter has at least one record of production will increase the genetic gain by 25 to 70 per cent, depending on the relative economic values of type and production.

The fraction of the intra-herd variance in average production (average of two records per cow) that could be attributed to yearly changes in the general environment of that herd was only 4.1 per cent. Such yearly changes are only minor obstacles to accuracy in selections. The yearly effects on production were largely eliminated by using the average of all records available on the cows studied.

Two selection indexes were developed for type and production from the estimates of the necessary parameters observed in this study. One of these gives type one-third as much attention as production, while the other gives both charac-

ters equal attention. Only information from the phenotypes of the cow and her dam were considered in constructing the indexes. Several of the combinations of information on the cow and her dam were found to yield progress about half as fast (R_{IA} is about 0.5) as if the exact Mendelian genotype of the cow were known.

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TOXICOLOGICAL EFFECTS OF TOXAPHENE ON DAIRY COWS

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The effectiveness and wide use of chlorinated hyydrocarbons as insecticides, not only on the bodies of dairy cows but on the forage crops which they consume, have resulted in considerable interest in the toxicity of these materials upon dairy animals. Considerable work has been done with DDT, and there is ample evidence that DDT is excreted in the milk of dairy cows sprayed with this material. Much less information is available on the newer chlorinated hydrocarbon insecticides. The research reported in this paper was limited to the study of the toxicological effects of toxaphene (chlorinated camphene) because this insecticide is being widely used in the control of grasshoppers on forage and pasture crops and in the control of flies, lice and ticks on cattle.

Studies reported by workers in the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture (6, 7) indicate that toxaphene compares favorably with lindane, methoxychlor and DDT in the control of horn flies and probably is equal to DDT and BHC in the control of the lone star tick. The Bureau (1, 8) also has studied the toxicological effects of toxaphene on cattle and found that, while older animals could be sprayed with heavy concentrations of toxaphene without toxicity symptoms, young calves were more susceptible and some toxic symptoms developed when calves were sprayed with sprays containing 1 per cent toxaphene. However, no accumulative toxic effect was observed in calves sprayed repeatedly with 0.75 per cent toxaphene. Milk from cows sprayed with 0.5 per cent toxaphene in both xylene emulsions and in wettable powder suspensions failed to show evidence of toxaphene when analyzed by the organic chlorine method. Workers at the Montana Experiment Station and the Hercules Powder Co. (4) have reported an increase in the organic chlorine content of tissues of beef animals fed alfalfa hay which had received applications of 4 lb. of toxaphene per acre. Unpublished work at this station indicated that the organic chlorine content of milk from cows fed 0.5 g. of toxaphene did not increase above normal.

The studies reported in this paper were conducted in cooperation with the Hercules Powder Co., Wilmington, Del., and the Departments of Biochemistry and Nutrition, Entomology, Dairy Husbandry and the School of Veterinary Medicine of the Texas Agricultural Experiment Station.

EXPERIMENTAL PROCEDURE AND RESULTS

The experiment was divided into three major phases: (a) Feeding high levels of toxaphene to dairy cows to determine the toxic level of the insecticide; (b) feeding calves milk from cows fed toxaphene and from cows sprayed with toxa-

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phene sprays in what was considered normal amounts; and (c) the analysis of milk samples taken from groups of cows sprayed with toxaphene sprays in concentrations required for adequate tick control.

In the first phase of the experiment, four Jersey cows were fed toxaphene in wettable powder administered in daily oral doses varying from 2.5 to 37.5 g. of actual toxaphene a day. These quantities amount to approximately 6.3 and 93.8 milligrams per kilogram of body weight. Table 1 shows the number of days each cow was fed various quantities of toxaphene in terms of daily dose and in terms of milligrams per kilogram of body weight. None of these cows showed any symptoms of toxicity as long as the daily dose of actual toxaphene was held to 5 g. per cow. When the dosage was increased to 7.5 g., typical symptoms of

TABLE 1

Oral feeding of toxaphene to Jersey cows

Cow no.	Daily dose		No. of days fed	Post-mortem organic chlorine content of omental fat	Remarks
	(*g.*)	(*mg./kg.*)		(*ppm.*)	
786	2.5	6.3	46		
	10.0	25.0	14[a]	67	Died[a]
789	2.5	6.3	46		
	10.0	25.0	4[b]		
	7.5	18.8	35[c]	126	Slaughtered[c]
768	5.0	12.5	52		
	7.5	18.8	59		
	15.0	37.5	1[d]		
	17.5	43.8	1		
	37.5	93.8	2[e]	160	Died[e]
776	5.0	12.5	121[f]	160	Died with Bloat[f]

[a] Developed toxic symptoms after receiving the 10-g. doses for 10 d. and died on the 15th day after the larger doses were started.

[b] Developed toxic symptoms after receiving 10-g. doses for 4 d. and appeared near death. Completely recovered when the toxaphene feeding was omitted for 1 day.

[c] Severe toxic symptoms were observed during most of the 35-d. period. Cow was killed for post-mortem studies; carrying a normal 4-mo. fetus when slaughtered.

[d] Severe toxic symptoms had been apparent for 31 d. before the daily doses were increased.

[e] Died in convulsion 36 hr. after the last dose of toxaphene.

[f] Died with bloat from unexplained causes; carrying a 6-mo. fetus at death.

toxaphene toxicity became evident, usually within 1 to 2 wk. The toxicity symptoms observed included extreme nervousness, evidenced by licking the ground and their feet, refusal to lie down, frequent defecation, loss of appetite and fits of running into fences, buildings and other cattle. Convulsions occurred at varying intervals of 24 hr. or more. The toxaphene was administered in gelatin capsules at 5 p.m. each day, and the most severe symptoms usually were observed before 8 a.m. By afternoon, symptoms of nervousness generally decreased. When more than 7.5 g. of pure toxaphene were fed daily, the convulsions became more frequent and severe; but even in the case of cow 768, which received very large doses, there was some evidence of recovery each afternoon. Number 768 finally died at 5 a.m., almost exactly 36 hr. after the last 37.5 g. dose was administered.

Omental fat samples from all four of these cows were analyzed for organic

chlorine content, and the results are shown in table 1. An organic chlorine content of 5 ppm. in the omental fat probably is about normal for cattle although some laboratories have reported finding as high as 15 ppm. of organic chlorine in the fat of untreated cattle.

Excessive decomposition prevented post-mortem studies of the tissues from no. 786. No gross or histological evidences of toxicity were found in the body of cow 776. The usual abnormalities associated with bloat were observed in her body, but the real cause of the bloat which was responsible for her death was not determined. Grossly, very few changes were observed in the organs of no. 789 and 768, but histologically a number of abnormalities were detected in the tissues. The really significant pathological changes were: (a) severe toxic degenerative changes in the liver, chiefly fatty degeneration, (b) similar toxic injury to the tubules of the kidney with moderate glomerular damage and early albuminuria, (c) cerebral degenerations and hemorrhages. These four cows were not in production during the experiment; therefore, no analysis of their milk was made.

In the second phase of the experiment, two Jersey cows in production were fed 0.5 g. of pure toxaphene a day, because it had been estimated that a dairy cow consuming average amounts of roughages, all of which had been sprayed with the normal amounts of toxaphene required for adequate grasshopper control, would consume not more than 0.5 g. of actual toxaphene in 24 hr. The milk from these two cows was fed to young Jersey and Holstein calves from birth to 30 days of age. All eight calves receiving the milk were slaughtered, along with four control calves which had received milk from the general herd. Gross and histological examination of the tissue from the calves revealed no abnormalities.

In the third phase of the experiment, eight Jersey cows were sprayed every 2 wk. over a period of 3.5 mo. with 2 to 3 qt. of spray containing 0.5 per cent toxaphene prepared from a wettable powder concentrate. Eight other cows were sprayed on the same days with pyrethrum sprays which contain no chlorinated hydrocarbon insecticide. Milk samples for analyses were taken 24 hr. after the first spraying, and every second day, for a period of 2 wk. thereafter. At the end of that period, limited laboratory facilities made it necessary to change the frequency of sampling to every fourth day. Duplicate samples were taken every second time for duplicate analysis. Each sample consisted of a 235-ml. composite sample taken from the evening and morning milkings, 12 hr. apart. The samples were analyzed for toxaphene by the organic chlorine method which consisted essentially of the extraction of the toxicant from the milk sample using 75 per cent ether - 25 per cent heptane as the solvent, decomposition of the organic chlorides in the extract by sodium-isopropanol treatment and finally, direct amperometric titration of the chloride ion with standard $AgNO_3$. This procedure is very similar to that used by Carter (3) to determine the DDT content of milk. Complete details are given in the *Toxaphene Manual of Analytical Procedure* prepared by the Hercules Powder Co. (5). All data are expressed as parts per million of organic chlorine in milk and may be converted to the equivalent amount of toxaphene by dividing by the factor 0.68. The analysis of the samples from the control cows of course permitted adjustment or correction for naturally occuring organic chlorides.

About 3 mo. after the last spraying with toxaphene in wettable powder, four of the same cows were sprayed with a water emulsion spray containing 0.5 per cent toxaphene prepared from an emulsifiable kerosene concentrate. These cows were sprayed every 2 wk. for a period of 2 mo. Four other cows also used on the other experiment were sprayed with the activated pyrethrum sprays on the same dates. Their milk was sampled and analyzed in the same manner described above. Figure 1 is a graph of the average quantities of organic chlorine found in the milk before spraying and between sprayings. Obviously there was no significant difference in the organic chlorine content of the control cows and those sprayed with toxaphene sprays prepared from wettable powder, but the organic chlorine content of the milk from the cows sprayed with the toxaphene spray prepared from the emulsifiable kerosene concentrate rose sharply within the first

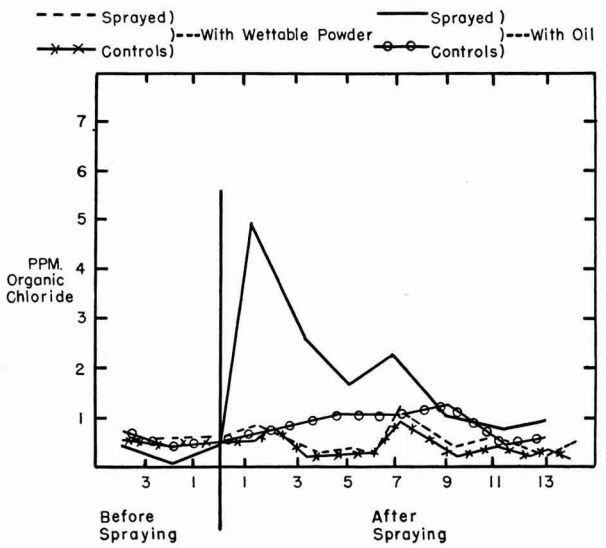

FIG. 1. Effects of spraying jersey cows with 0.5% toxaphene in sprays prepared from wettable powder and emulsifiable oil concentrates upon the organic chloride content of the milk.

24 hr. after spraying and then gradually declined over a period of 1 to 2 wk. The t value of the mean difference between the organic chlorine values of the two groups (control and experimental) in the emulsifiable oil experiment obtained from milk sampled 1 day after spraying was 8.42 with 14 d.f. In the wettable powder experiment one of the greatest mean differences in the organic chlorine content of the milk also was on the first day after spraying, but the t value was 1.06 with 45 d.f.

CONCLUSIONS

From the limited information available, it appears that a Jersey cow can consume 5 g. of toxaphene a day without harmful effects. Daily consumption of larger quantities, even for a period of 2 to 3 wk., is likely to cause severe toxicity or death.

If forage is sprayed in the quantities normally required for grasshopper control, it appears unlikely that dairy cows will consume enough of the insecticide to produce any toxic symptoms. Cows evidently recover very rapidly from large doses of toxaphene and therefore probably can consume a considerable quantity in any 1 day without harmful effect.

Calves are not harmed by drinking milk from cows fed 0.5 g. of toxaphene daily.

It appears safe to spray dairy cows with 0.5 per cent toxaphene prepared from wettable powder concentrates, but spraying with this concentration of toxaphene from emulsifiable oil concentrates apparently results in the toxaphene being excreted in the milk for 5 to 7 days after each spraying.

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PREGNANCY INTERRUPTION AND BREEDING TECHNIQUES IN THE ARTIFICIAL INSEMINATION OF COWS

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During recent years increased emphasis has been placed on the effect of inseminating techniques on the results of the artificial insemination of dairy cattle. Studies on this problem have shown that intracervical or intrauterine deposition of the semen by the rectovaginal technique results in higher fertility than the method involving the use of the speculum and deposition of semen just through the *os uteri* (6, 8, 13, 15). Studies comparing the effectiveness of different sites of semen deposition into the cervix and uterus by the rectovaginal technique have indicated little or no difference in conception rate (7, 14). However, the possibility of causing uterine infections by intrauterine insemination has been suggested (12). The fact that brucellosis easily can be transmitted by intrauterine insemination but is less easily transmitted by intracervical insemination of infected semen also has been reported (9, 10). None of these studies have considered the effects of depositing semen in different sites in the reproductive tract of cows that are returned to service though already pregnant from an earlier service.

Several investigators have reported the occurrence of estrus and the rebreeding of cows that had conceived to an earlier service (2, 3, 4, 5). In more than 1,000 natural matings of cows in Britain, Donald (4) found that 3.4 per cent of the total matings were to cows already pregnant. Branton (2) reported that 64 services (or 3.45 per cent) out of a total of 1,855 natural services over a period of years in the Louisiana State University dairy herd were made after conception had occurred.

By using vaginal smears, Mirskaja and Smirnov-Ugrjumov (11) concluded that 21.7 per cent of 92 cows showed estrus during pregnancy. This is a considerably larger proportion than shown by other workers whose data were based on actual matings. Consequently, the high values may be open to question on the basis of the reliability of the vaginal smear as an indication of estrus in the cow (1). Nevertheless, the data of the various investigators reported suggest that a sizeable number of cows already pregnant may be submitted for artificial insemination service.

Furthermore, the findings of Donald (4) indicated that estrus in pregnant cows occurred usually in the first 6 mo. and most frequently between the first and third month. This would make detection of the pregnancy more difficult, for diagnosis is not made easily on cows less than 60 days pregnant.

Field results have suggested that artificial insemination of the pregnant cow may result in the disruption of the pregnancy. Dyrendahl (5) has mentioned

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the problem of abortion following the artificial insemination of pregnant cows in Sweden. Still, the authors know of no controlled investigations which indicate the effects of artificial insemination on the pregnant cow.

The present investigation was designed to compare the effects of depositing diluted semen midway in the cervix and just through the cervix into the body of the uterus by the rectovaginal technique on the maintenance of pregnancy in cows.

EXPERIMENTAL

Twenty-one brucellosis-free cows ranging from the 64th to the 152nd day of pregnancy were divided into three groups so that the cows in each group were fairly comparable on the basis of the stage of pregnancy. All cows were inseminated with 1 ml. of diluted semen. The diluter used for the insemination of the first two groups of cows consisted of 2.9 per cent sodium citrate dihydrate-egg yolk containing 300 mg. sulfanilamide per 100 ml. A similar diluter was used for insemination of the third group except that 1,000 units of penicillin G sodium and 1,000 γ streptomycin sulfate per ml. diluted semen were added. The group of seven cows was inseminated by the rectovaginal technique midway into the cervix, with care being taken not to pass the inseminating tube farther through the cervix than the midpoint at any time during the procedure. The eight cows in the second group and the six cows in the third group were inseminated by passing the inseminating tube just through the cervix and depositing the diluted semen into the body of the uterus. Following the inseminations, the cows were confined and observed daily for signs of vaginal discharge, aborted fetuses or other abnormal conditions. The cows were slaughtered from 16 to 77 days after insemination, and post-mortem examinations of the reproductive tracts were made. Less time was required for resorptions in early pregnancy than in later pregnancy; therefore the times were varied between insemination and slaughter according to the stage of pregnancy at insemination. At the time of slaughter, the uteri and their contents were examined carefully. The crown-rump length of intact fetuses was measured, and the bones of the resorbed fetuses were collected.

RESULTS AND DISCUSSION

The group of cows that was inseminated midway into the cervix showed no abnormal conditions as a result of insemination, except a slight vaginal discharge which persisted in two cows for 3 days. At the time of slaughter the cervix of each was sealed with mucus, the placentas were intact and showed no signs of having been disturbed by the mid-cervical insemination. The fetuses also appeared normal, were intact and the crown-rump lengths corresponded closely with those reported for bovine fetuses of a comparable age (16). There was no indication that the mid-cervical insemination of these pregnant cows had altered the existing pregnancy (table 1).

The cows in which diluted semen without penicillin and streptomycin was inseminated through the cervix into the body of the uterus began showing a purulent discharge by the third day following the insemination. This discharge be-

came gradually worse and continued in every case until the calf was aborted or the cow was slaughtered. One of the eight cows in this group aborted 9 days after she was inseminated. When the remaining seven cows of this group were slaughtered 18 to 77 days after the insemination, each had an open cervix containing no mucous seal. In all seven cases the soft tissues of fetuses were either completely resorbed or badly disintegrated (table 1). The walls of the uteri were thickened and the mucosal linings were red or purplish in color. The bones of the fetuses were present in the uteri, along with a purulent fluid. The fetal placentas were no longer recognizable.

TABLE 1

Effect of site of semen deposition by artificial insemination on the maintenance of pregnancy in cows

Site of insemination and diluter	Cow	Day of pregnancy when inseminated	Fetus at slaughter		Fetal membranes
			Age	C–R length[a]	
	(*no.*)		(*d.*)	(*in.*)	
Mid-cervix CSAY[b]	1A	152	174	16.5	Intact
	2A	150	182	18.5	Intact
	3A	121	141	14.0	Intact
	4A	120	164	18.7	Intact
	5A	101	133	13.5	Intact
	6A	91	109	8.5	Intact
	7A	84	102	7.0	Intact
Body of uterus CSAY	1B	151	Resorbing		Disintegrating
	2B	149	Aborted (9 d.)		Disintegrating
	3B	132	Resorbing		Disintegrating
	4B	110	Resorbing		Disintegrating
	5B	104	Resorbing		Disintegrating
	6B	92	Resorbing		Disintegrating
	7B	72	Resorbing		Disintegrating
	8B	64	Resorbing		Disintegrating
Body of uterus CSAY plus penicillin and streptomycin	1C	152	185	22.0	Intact
	2C	141	161	14.0	Intact
	3C	124	147	16.0	Attachments partially destroyed
	4C	104	120	9.5	Intact
	5C	82	Resorbing		Disintegrating
	6C	69	92	6.5	Intact

[a] C–R = crown-rump length in inches.
[b] CSAY = citrate-sulfanilamide-yolk diluter.

In the third group of cows inseminated in the body of the uterus with diluted semen containing penicillin and streptomycin, pregnancy continued without interruption in four animals (table 1) and normal intact fetuses were found at slaughter. The two other cows of this group showed purulent discharges from a few days after insemination until slaughter. In one of these cows a dead, resorbing fetus with disintegrating fetal membranes was found. In the other approximately one-half of the placental attachments had been destroyed, but the fetus was still living.

It should be borne in mind that the pregnant cows used in these investigations were not showing estrus when they were inseminated. However, in every case

when sulfanilamide was the only bactericidal agent present, and in two out of six cases when penicillin and streptomycin were added, the pregnancy was interrupted by intrauterine insemination. In contrast to these results, mid-cervical insemination permitted the continuance of normal pregnancy. Thus, use of the mid-cervical insemination technique would diminish the dangers of pregnancy interruption in cases of improper cow identification which occurs occasionally in the field.

These findings, along with those of other investigators, strongly indicate the necessity for a re-evaluation of artificial insemination techniques. The reduced danger of causing uterine infections (12), the decreased possibility of transmitting brucellosis (9, 10), the diminished danger of interrupting a pregnancy and the ease of the procedure are all arguments in favor of mid-cervical insemination. These arguments, plus the fact that conception rates comparable with other present-day methods of insemination have been obtained (7, 14), point to intracervical insemination (rectovaginal technique) as the logical procedure in routine artificial insemination of dairy cattle today.

SUMMARY

A study was made comparing the effects of artificial insemination techniques on maintenance of pregnancy in cows (64th to 152nd day of pregnancy). Using semen diluted with sodium citrate-sulfanilamide-yolk, seven cows were inseminated by the rectovaginal technique with mid-cervical deposition of semen and eight cows with intrauterine semen deposition. All cows that received mid-cervical semen depositions continued normal pregnancies and had normal intact fetuses and fetal membranes when slaughtered 18 to 44 days later. Of eight cows inseminated just into the uterus, one aborted 9 days after the insemination. The remaining cows of this group had fetal resorptions, as manifested by a purulent discharge prior to slaughter and the presence of disintegrated fetuses and fetal bones upon slaughter 18 to 77 days following the insemination. In four of six cows that were inseminated into the uterus with diluted semen containing added penicillin and streptomycin, normal pregnancies continued, while pregnancy was interrupted in the other two. Therefore, mid-cervical deposition of the semen by the rectovaginal technique is recommended in the artificial insemination of cows.

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LACTATION STUDIES. I. EFFECT OF GESTATION[1]

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Inhibition of lactation due to advancing gestation starts sometime after the fifth month (1, 2, 4, 5). Ragsdale *et al.* (1), using Guernsey Advanced Registry records, observed a reduction of 480 to 800 lb. of milk in the lactation record as pregnancy during lactation exceeded 5 mo. Gowen (5), also using Guernsey Advanced Registry records, estimated the decline in the lactation record attributable to pregnancy at 400 to 600 lb. of milk. There was no influence on butterfat percentage. Gaines and Davidson (2), using the same source for data, estimated the decrease to be 2.5 lb. of 4 per cent fat-corrected-milk (hereafter referred to as F.C.M.) for the first 5 mo. of pregnancy and 256 lb. of F.C.M. for the gestation period (9.2 mo.). Gooch (3), using Jersey monthly test records, noted only a slight decline in lactation, starting the seventh month of pregnancy. Sikka (6) found monthly variations accounted for 9.57 per cent of the total variation in persistency of Ayrshire cows. Gaines and Davidson (2) reported that younger cows were more persistent milkers than older cows.

Studies on persistency (6) have indicated that high initial yields were approximately three times more important than persistency in determining total yield. The inhibiting effects of advancing pregnancy on lactation appear to vary with age, level of production, month of calving and breed.

PROCEDURE

Production records on 82 cows (Holsteins, Guernseys and Jerseys) made from October 1946, to August 1950, in the State College of Washington dairy herd were used. This period was selected because environmental differences were known to be minimal. The cows had no access to pasture during this period but did receive green feed during May and June of 1949 and 1950. The cows were fed for near maximum production throughout the period. The records were classified according to breed, age, calving interval, times milked per day and month of conception during the lactation period. The data were punched on I.B.M. cards and sorted for final analysis. The statistical analyses used were essentially as outlined by Snedecor (7).

RESULTS AND DISCUSSION

Daily milk weights and butterfat tests were available for the first 223 days of pregnancy for eight first-lactation Holsteins. Three were milked twice daily,

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and five were milked three times daily. These animals all calved during November and December, 1947, and conceived during February and March, 1948.

Production for 60 days prior to conception, including day of conception, also was studied. Preliminary survey of the data indicated the desirability of studying the lactation curves in segments. These divisions are apparent in figure 1. The between-days variation, after removing variance attributable to lactation decline, was non-significant for milk, butterfat yield and F.C.M. The daily variation for fat test was highly significant. There was no significant difference

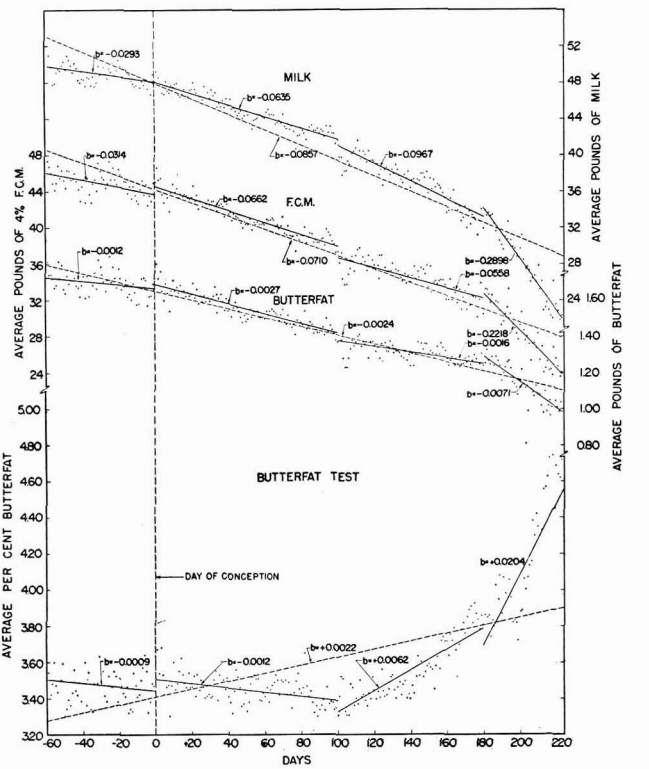

FIG. 1. The effect of advancing pregnancy on yield of milk, F.C.M., butterfat and daily butterfat test.

in milk and fat yield between cows milked two and three times daily with respect to rate of decline in lactation at the various periods after pregnancy. However, the cows milked three times daily increased in fat test a little more rapidly during the lactation-inhibiting phase, *i.e.*, after 180 days of pregnancy, thereby causing a significant difference between the two groups of cows in butterfat test and F.C.M. yield. The patterns for average daily yield of milk, F.C.M., butterfat and butterfat test are shown in figure 1 along with the linear regression values for each segment.

Milk and F.C.M. declined at essentially the same rate during the first 100

days of pregnancy. However, during the next 80 days, F.C.M. and butterfat yield declined at a slightly slower rate due to increasing butterfat test, whereas milk yield declined approximately one-third faster.

From 181 to 223 days of pregnancy, decline in production was three to four times faster than the previous period. This was true for butterfat and F.C.M., even though butterfat test was rising rather rapidly during this latter period. The daily variation in fat test was most noticeable during the preconception period and became only slightly less variable during the first 100 days following pregnancy. After 100 days of pregnancy, fat test increased gradually for approximately 40 days and rapidly thereafter. A very interesting feature of figure

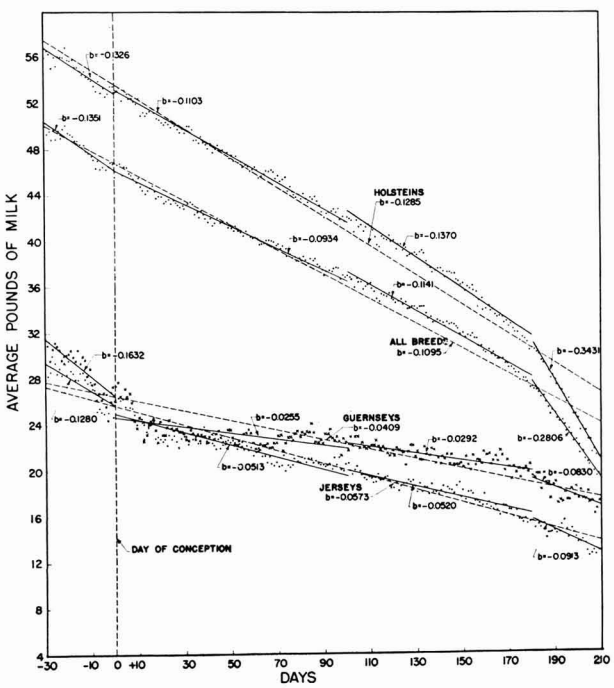

FIG. 2. Effect of advancing pregnancy on milk yield of Holstein, Guernsey and Jersey cows.

1 is the rather definite cycles shown in the production of milk and butterfat throughout the period under study. These cycles resemble changes that have been noted during the estrual cycle (8). The close similarity of changes in milk and F.C.M. yield of these eight cows made it feasible to extend the data to other cows for which only daily milk yields were accurately known.

Fifty-three such records were tabulated, representing 40 Holsteins, eight Jerseys and five Guernseys. The cows used had calving intervals from 12 to 18 mo. and were in lactation a minimum of 210 days after pregnancy. The summary, as shown in figure 2, reveals one marked difference between the breeds.

Both the Jerseys and Guernseys dropped rapidly during the first 10 post-conception days. Just how typical this may be, however, is open to considerable question, since too few cows are involved. Inhibition of lactation due to gestation becomes noticeable between 170 and 180 days in all three breeds. The cyclic nature of milk yield following pregnancy, as mentioned earlier, is quite pronounced for the individual breeds and for all breeds. With this number of records involved and no other arrangement other than day of conception (which occurred as much as 180 days later in lactation in some cows than others) it would seem this phenomenon has the beginning of pregnancy as the focal point.

The rate of lactation decline for Holsteins during the first three periods was essentially the same. However, rate of decline after 180 days of pregnancy was 2.5 times faster. The Jerseys and Guernseys declined in lactation very slowly after pregnancy, but the rate of decline after 180 days increased 1.8 times for Jerseys and 2.8 times for Guernseys. The loss in milk yield attributable to pregnancy 30 days beyond 180 days would be 213, 16 and 24 lb. for Holsteins, Jerseys and Guernseys, respectively, or 2.1, 0.3 and 0.5 per cent of the total production during the 240-day period under study. Thirty-seven and 16 cows, respectively, had calving intervals between 12 to 14 mo. and 14 to 18 mo. As shown in the upper portion of figure 3, delayed pregnancy has little or no effect on the time advancing pregnancy caused a distinct inhibition of lactation. The portion of variance attributable to days by calving interval was non-significant. Possible differences due to number of times milked are shown in the lower portion of figure 3. Thirty-two and 21 cows, respectively, were milked three and two times daily. Cows milked three times daily showed higher average production during the first half of the study but declined at a more rapid rate, so that average yield was essentially the same during the latter half. The two groups of cows showed inhibition of lactation at approximately the same time following pregnancy.

Thirty of the 40 Holstein cows used in the study had calving intervals between 12 and 14 mo. This group was selected to study differences due to age, average productive level and month of conception. Since times milked per day had been shown by two previous analyses not to influence the changes associated with advancing pregnancy, separate breakdowns on this variable were not used. These 30 cows were classified according to average milk yield during the 240-day period studied. Cows averaging 38 lb. or less were considered low producers (eight cows); those between 38 and 48, average producers (16 cows); and those above 48, high producers (six cows). The curves for these groups of cows are shown in figure 4. Cows in the low-producing group declined at only one-half the rate during the first 100 days of pregnancy, as compared with either the average or high producers. The group differences in rate of decline were less from 101 to 180 days of pregnancy, but the low-producing cows declined in production approximately one-third slower than either the average or high producers. The decline was not greatly different from 101 to 180 days for all three groups. After 180 days, the high producers declined three times the rate of the previous 80 days as compared with 2.5 and 2 times, respectively, for the average

and low producers. Assuming pregnancy was the sole cause for the decline during the 30 days beyond 180, the error in total production during the 240-day period would be 0.42, 0.78 and 0.82 per cent, respectively, for the low, average and high producers. By analysis of variance, these differences in rate of decline

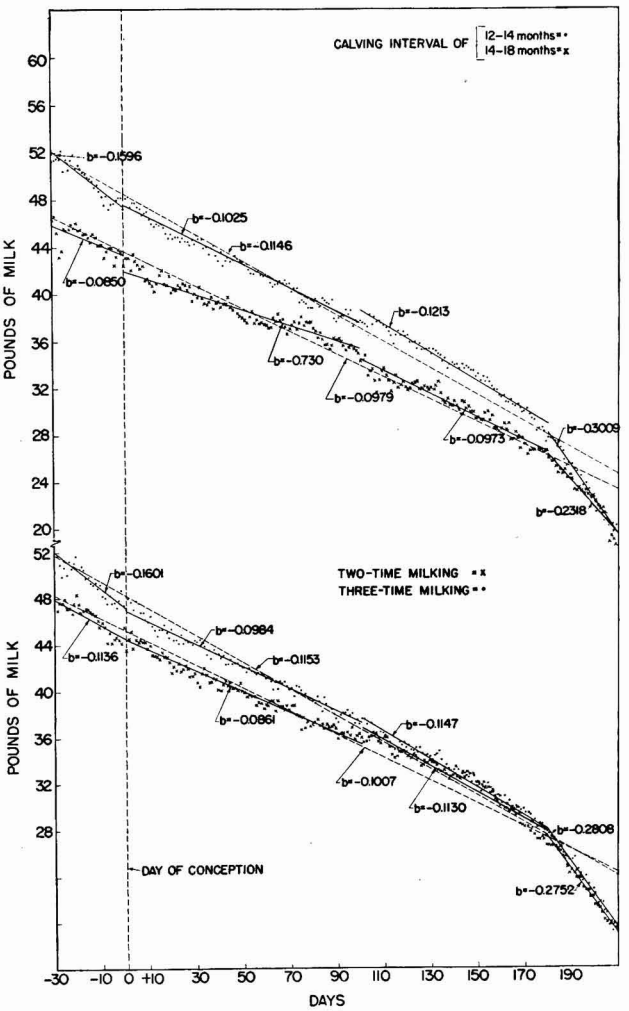

FIG. 3. Effect of calving interval and times milked per day on changes in milk yield following start of pregnancy.

by productive levels were shown to be highly significant. The variation by days by productive level was non-significant when the variance attributable to days and productive level was removed. Thus, productive level had no influence on the changes attributed to advancing gestation, but the three groups responded at varying intensities.

Since productive level and age are interrelated, particularly in cows in the same herd, an analysis of age differences was made as shown in figure 5. There was a distinct tendency for the 2- and 3-yr.-old cows (first and second lactation) to decline more slowly in milk yield than older cows. There were seven, six, seven and ten records, respectively, in the 2-, 3-, 4- and over 4-yr.-old age groups.

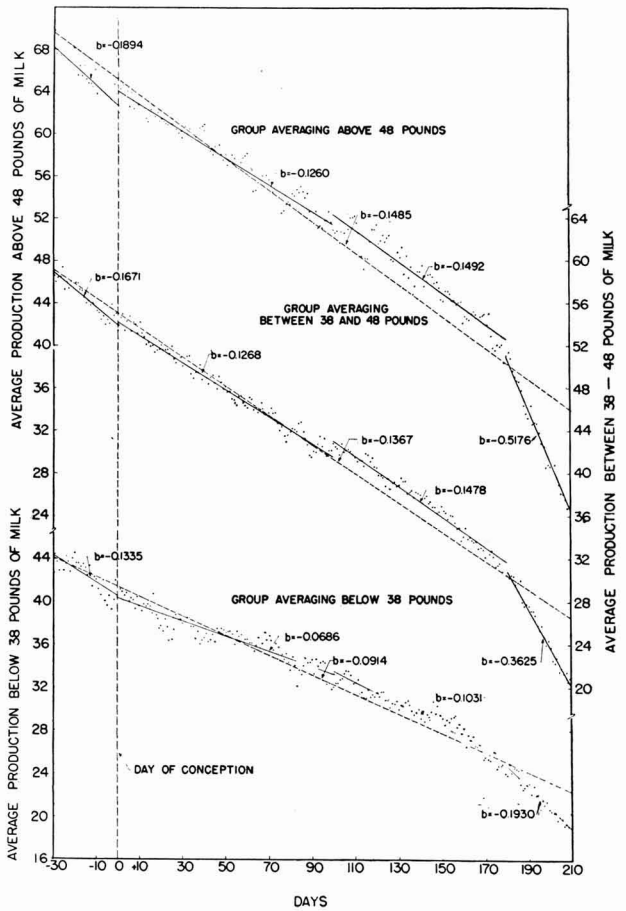

FIG. 4. Relation of level of milk production to rate of decline after conception in 30 Holstein cows with calving intervals of 12–14 months.

Approximately 45 per cent of the variance attributable to age could be reflected in production level, with another 52 per cent, possibly due to month-of-conception differences. Actual differences due to each of the three variables cannot be separated accurately in these data, due to too few observations. Each of these variables contains variance due to the other two. However, even when these variances were deducted from age variance, the rate of lactation decline was still

significantly less in younger than in older cows. The differences in response between days by ages were non-significant when variance attributable to age and days was removed. Here again yield changed in the same direction, but at a significantly different intensity. The errors in yield attributable to inhibition of pregnancy from 181–210 days were 0.20, 1.03, 0.96 and 0.89 per cent, respectively, for 2-, 3-, 4- and over 4-yr.-old cows.

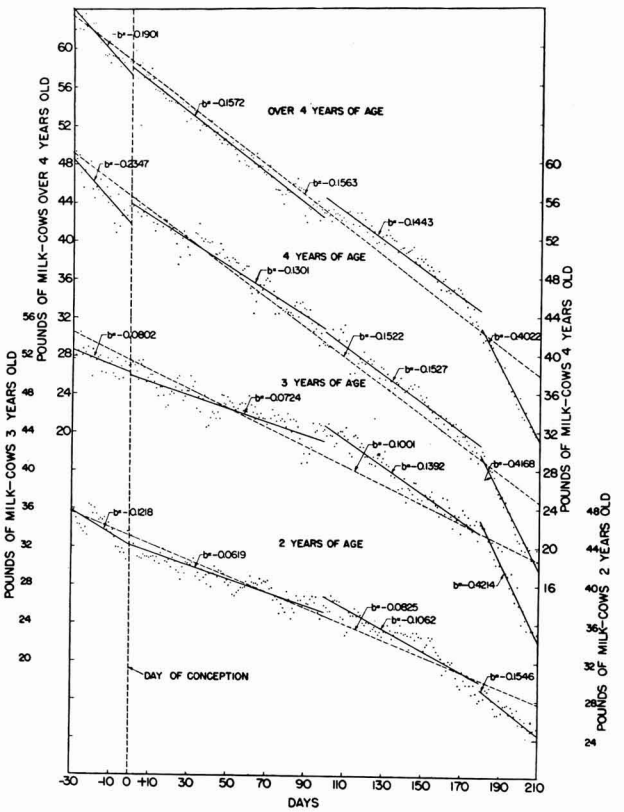

FIG. 5. Relation of age to decline in lactation as affected by advancing gestation. (30 Holstein cows with calving intervals of 12–14 months.)

The data also were sorted by months of conception. This analysis (figure 6) shows extreme variation in rate of decline in lactation between the several groups of months plotted. The rate of decline after 180 days of pregnancy was nearly twice as fast for cows conceiving during April and May as compared with June and July or December and January. The average level of production was not greatly different between the various groups, although the five cows conceiving during June and July had initially higher production. The error attributable to month of concepiton for gestation from 181 to 210 days was 0.53, 0.76, 1.20,

0.45, 1.24 for December and January, February and March, April and May, June and July and August through November, respectively. The differences were highly significant, but days by month groups were non-significant. From these data it would appear that far greater error occurs from effects due to season of freshening when measuring the producing ability of dairy cows than from inhibition of lactation by pregnancy beyond 180 days. The two variables are compensating, since cows with most rapid declines—presumably due to season of

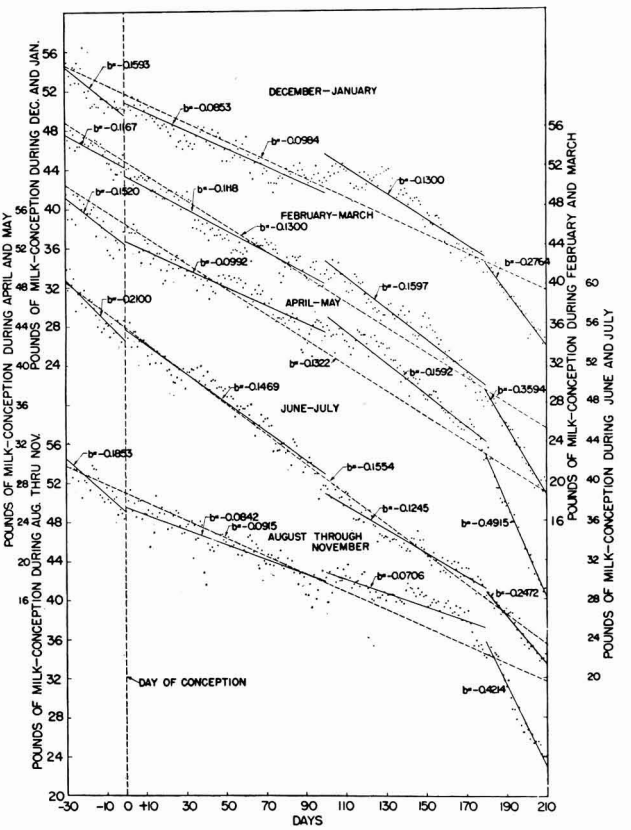

FIG. 6. Effect of month of conception on lactation decline of 30 Holstein cows with calving intervals of 12–14 months.

freshening—have lower error due to inhibition of lactation by advanced gestation.

Data on ten cows (eight Holsteins, one Jersey and one Guernsey) averaging over 5 yr. of age, that were open during a 365-day lactation period are summarized in the lower portion of figure 7. The average decline in yield of these cows was 0.1134 lb. per cow per day for the 365-day period. For a comparable 240-day period, the average decline was 0.1292 lb. per cow per day, which is considerably slower than the 0.1563 lb. per cow per day decrease for 10 cows over

4 yr. of age that carried calves 210 days of the lactation period. This difference was partially due to flattening out of the lactation curve of the non-pregnant cows during the latter part of the lactation. The average fat tests (taken once monthly) for the non-pregnant cows increased at a rather uniform rate throughout the 365-day period. This is in contrast to the rapid rise during the last portion of the lactation curves of cows presented in figure 1.

A normal lactation curve for 19 Holstein cows, 10 of which were milked three times daily and nine milked two times daily, is presented in the center portion of figure 7. Daily butterfat tests also were available and are presented as consecu-

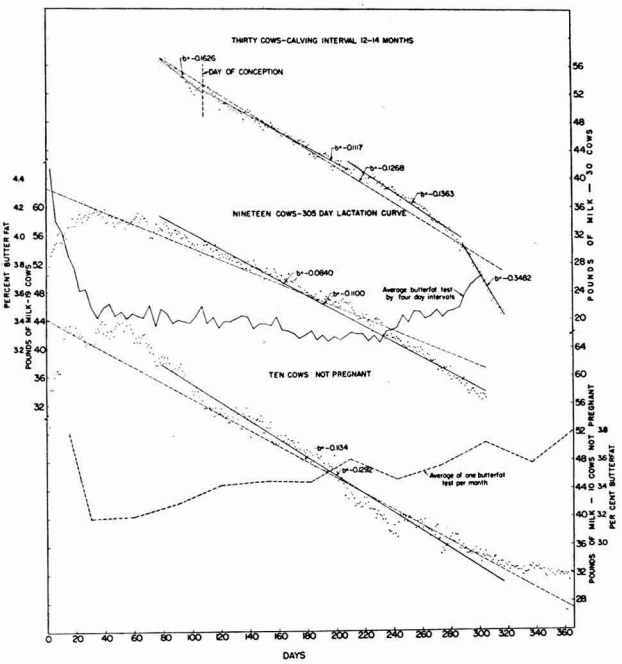

FIG. 7. Comparison of lactation decline of 10 non-pregnant cows to performance of other cows used for this study.

tive 4-day averages for the 305-day period. Eight of the 19 cows carried their calves 223 days (fig. 1). The other 11 carried calves 180 days or less and all were over 3 yr. of age at freshening.

The lactation curve for milk for the 10 non-pregnant cows was considerably steeper from 60 to 120 days after the start of lactation as compared with the 19 cows, but tended to decline at a slower and slower rate after 240 days in lactation, whereas the 19 cows in all stages of pregnancy showed an increased rate of decline from 240 to 305 days. Eight cows in this latter group were carrying calves 180 days or longer.

The summary for the 30 cows used for data presented in figures 4, 5 and 6 is shown in the upper portion of figure 7. These cows conceived, on the average,

108 days after the start of lactation, and the data are presented in the same relative position as the other data presented in figure 7. The lactation decline for the 240-day period averaged 0.1268 lb. per cow per day, as compared with 0.1100 and 0.1292 lb., respectively, for a comparable 240-day period for the 19 cows in various stages of pregnancy and the 10 non-pregnant cows (figure 7).

SUMMARY

A total of 82 production records (Holstein, Guernsey and Jersey breeds) were utilized to study the effect of gestation on yield. The cows were fed and managed for near-maximum yield and did not have access to pasture during the period studied.

Daily milk weights and butterfat tests were available for 19 Holstein cows, eight of which carried calves a minimum of 223 days. In the latter group, five were milked three times daily and three were milked twice daily. There was no difference in rate of decline in lactation between the two groups (milk, F.C.M. and butterfat) with advancing pregnancy. The rate of decline in yield was three to four times faster from 181–223 days of pregnancy as compared with 101–180 days of pregnancy. Butterfat test was more variable during preconception periods and progressively less variable as length of gestation increased.

Milk records for 53 cows milked two and three times daily and carrying calf for 210 days revealed that inhibition started at approximately the same time regardless of the number of times milked daily, age of the cow, breed, month of conception, production level of the cow or calving interval up to 18 mo.

A lactation curve for 10 cows not pregnant during the first 365 days in lactation revealed a tendency for rate of decline in milk yield to slow after 330 days. This is in contrast to an accelerating rate of decline in cows pregnant more than 180 days. Lactation decline appears to follow a cyclic pattern following conception not unlike that observed for estrual cycles.

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LACTATION STUDIES. II. EFFECT OF ESTRUS[1]

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Paper I of this series (2) reports variations in lactation that are associated with pregnancy. Even after conception, there was a pronounced cyclic fluctuation in the yield of milk and butterfat not unlike those associated with the estrous cycle as reported by Morrison *et al.* (7). Hooper (3, 4) reported much earlier that some cows showed considerable variation in yield at the time of heat, while others showed little or no variation. Hooper and Brown (5) studied records from 29 cows and found an average decrease of 0.1 lb. of fat and 1.5 lb. in milk production on the day of most evident heat. McCandlish (6) compared milk production 3 days preceding and following the day of breeding on 868 cases. Milk production was 4 per cent higher 2 days before breeding than on the day of breeding. Milk production the day after breeding was higher than the day of breeding but lower than the other 4 days studied. Copeland (1) made 211 comparisons from Jersey Register of Merit records based on 1-day supervisors' reports. Milk yields were compared for 2 days preceding and following supervision. An average decrease of 0.63 lb. of milk on day of heat was observed. The variation was less than 2 lb. per day in 75 per cent of the cases. Butterfat percentage increased an average of 0.1 per cent. Milk yield 2 days preceding heat was higher in 112 cases and lower in 99 cases than yields 2 days following heat.

PROCEDURE

Daily milk and butterfat yields were determined on 19 Holstein cows in the State College of Washington herd. Ten cows were milked three times daily and had a total of 38 normal estrous cycles, as compared with 42 for nine cows milked two times daily. Milk, butterfat and 4 per cent fat-corrected-milk (F.C.M.) yields and butterfat tests were recorded for 8 days preceding and 11 days following estrus. This resulted in 20-day observation periods, counting the day of estrus.

In addition, 703 estrous cycles occurring during the years 1946–50 in the Jersey, Guernsey and Holstein breeds were studied. Only daily milk yield was accurately known for this latter group of records. The data were classified according to breed, age, times milked, season, length of cycle, number of heat periods during current lactation period and stage of lactation during which the heat period occurred. Records also were obtained for seven cases of questionable estrus and 36 cases falling midway between observed 42- to 46-day cycles. I.B.M.

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[2] Acknowledgment is made to W. T. Southwarth for assistance with the I.B.M. analysis of the data.

was used for tabulating the data. Methods of statistical analysis were essentially as outlined by Snedecor (8).

Environmental influences during the period were minimum. The cows were fed for near maximum production and had no access to pasture. Some green feed was provided the latter part of May and during June of 1948–1950. Cows in heat were separated from the rest of the herd as soon as heat was observed. This was very prompt in each case, since exercise lots are around the dairy barn and can be seen from each wing of the barn. Detailed health records on each cow made it possible to omit data during periods of illness.

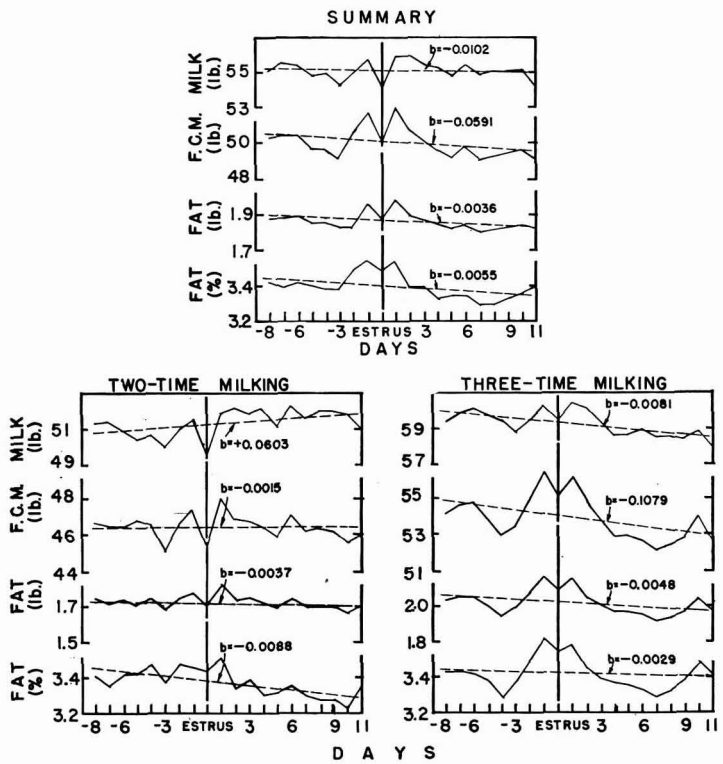

FIG. 1. Yield of milk, F.C.M., butterfat and per cent butterfat for 80 estrous cycles of 19 Holstein cows milked two and three times daily.

RESULTS AND DISCUSSION

Variation in milk, F.C.M., fat and per cent fat of 80 estrous cycles from 19 Holstein cows. Nine cows milked two times daily had 42 heat periods, and 10 cows milked three times daily had 38 heat periods, from freshening to conception. Every milking was individually weighed and tested for butterfat content. In the analysis for this study, daily totals were used.

Figure 1 indicates the cows milked three times daily showed more daily varia-

tions for F.C.M., butterfat and butterfat test than cows milked twice daily. Milk yield was about equally variable and showed essentially the same type of fluctuations except for the day of estrus. The 42 cycles for cows milked twice daily produced an average of 1.9 lb. less the day of heat than expected, as determined by the method of least squares. The average milk yield for 38 cycles for cows milked three times daily exceeded the expected by a small margin.

More estrous periods for cows milked twice daily occurred during the inclining stage of lactation which lasted throughout the first 50 days, as compared with only the first 30 days for the ten cows milked three times daily. Both groups of cows produced more milk the first 3 to 4 days after heat than any other day of the 20-day period under study, except the day preceding estrus for cows milked three times daily. Butterfat test generally was higher for 5 days centered around estrus than any other period of the cycle. By analysis of variance, it was shown that the daily variation was highly significant for milk, F.C.M., butterfat and butterfat test. The variance attributable to differences between days × number of daily milkings also was highly significant for milk, F.C.M., butterfat and butterfat test.

The variations of yield and fat test on the day of estrus for milk, F.C.M., fat and fat test were 98.3, 99.7, 100.9 and 102.5 per cent, respectively, of the expected for 19 cows having 80 estrous cycles. The greater decline in yield the day of estrus for two-time milking as compared with three-time milking may be largely related to udder capacity. Most cows show lower production for one milking when in estrus. This appears largely due to incomplete "let down," since an above average quantity is given at the next milking. Udder capacity is more likely to be the limiting factor for high producing cows milked twice daily than for high producing cows in the same herd milked three times daily. Fat test was consistently higher for the 5 days centered around estrus.

Effect of stage of lactation. Four divisions of the data were made, based on stage of lactation when estrus occurred. The results as shown in figure 2 indicate no considerable difference in trend lines for estrus occurring at one stage of lactation or the other. However, greater variability in yield was apparent after 100 days of lactation. During the first 100 days of lactation, yields exceeded expected on the day of heat, whereas after 100 days in lactation, decline was considerably greater the day of estrus as compared with the day before and the day after. Yields of milk, F.C.M. and fat were consistently lower 3, 4 and 5 days before estrus than during any other period, with the exception of 8 days after estrus.

From these data, production records for milk and fat based on one test per month are likely to be too high for cows tested around the time of heat, rather than too low. This is particularly true for the first 100 days of lactation. Most cows are pregnant again by this time. In figure 2, average milk yield for 101–150 days was increasing instead of decreasing. This cannot be readily explained, since rate of decline was 0.0776 lb. per day for estrous cycles occurring after 150 days in lactation (averaged 203 days). This latter rate of decline is essentially the same as that observed for the 19 cows during this same period (2).

The significance of daily variation within stage of lactation was also tested. The results are shown in table 1.

Variation in milk yield by breeds. A total of 639 estrous cycles exceeding 18 days in length and representing 89, 143 and 407 cycles for the Guernsey, Jersey

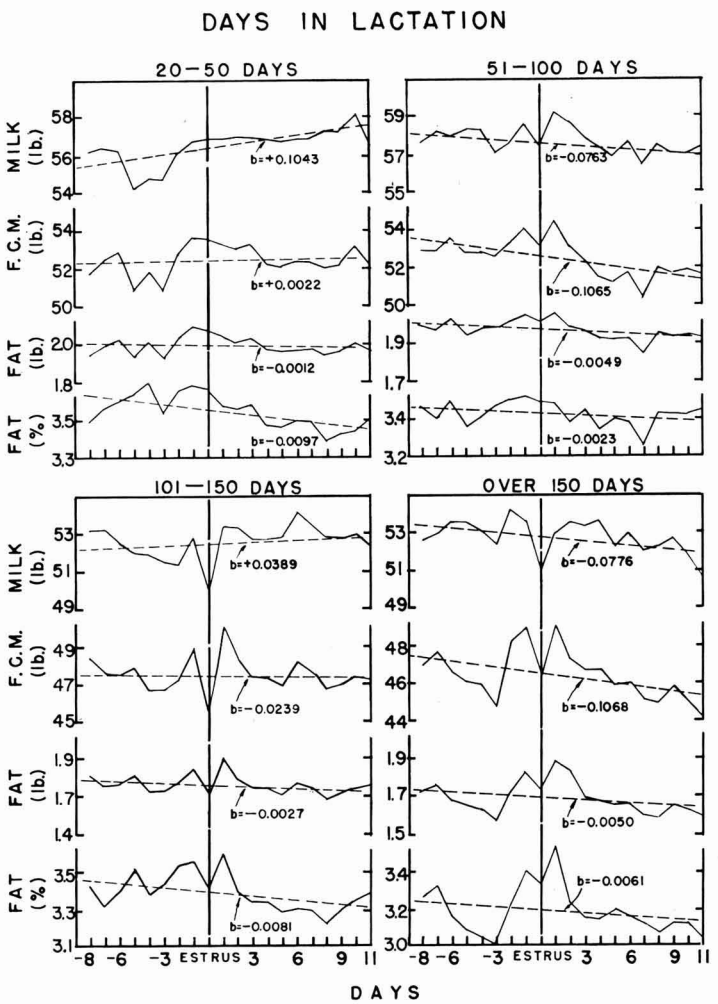

FIG. 2. Relation of stage of lactation to changes in yield.

and Holstein breeds, respectively, was available for this study. The summaries for breeds as shown in figure 3 reveal essentially the same pattern of variation for each of the three breeds. However, the Jerseys were lower in production the day before and day of estrus, and higher the day after estrus, than were Holsteins or Guernseys.

TABLE 1

Probability level attained by daily variation within stage of lactation

Stage of lactation	Error degrees of freedom	Probability level:			
		Milk	F.C.M.	Fat	Fat test
(*d.*)					
20– 50	266	0.05	0.05	0.05	0.01
51–100	475	0.05	0.01	0.01	0.05
101–150	361	0.01	0.01	0.01	0.05
Over 150 days	266	0.05	0.01	0.01	0.05

Variation in milk yield by stage of lactation. The 639 estrous cycles presented in the previous section were analyzed further by stage of lactation. Analysis of variance of results shown in figure 4 indicated a highly significant difference between days by stage of lactation. The breed × days × stage of lactation variance was non-significant. Hence, breeds were combined for presentation of data. Thirteen heat periods occurred between 11 and 20 days in lactation when lactation rate was increasing rather rapidly. The period around the time of estrus shows a distinct increase in yield over expected, or possibly an inhibition of milk yield during the latter portion of the luteal phase. Since subsequent stages of lactation studied do not bear out the possibility of distinct inhibition during the luteal phase, these data are interpreted to indicate that the early follicular phase (3 or more days before heat) is more likely a lactation inhibitor. This becomes

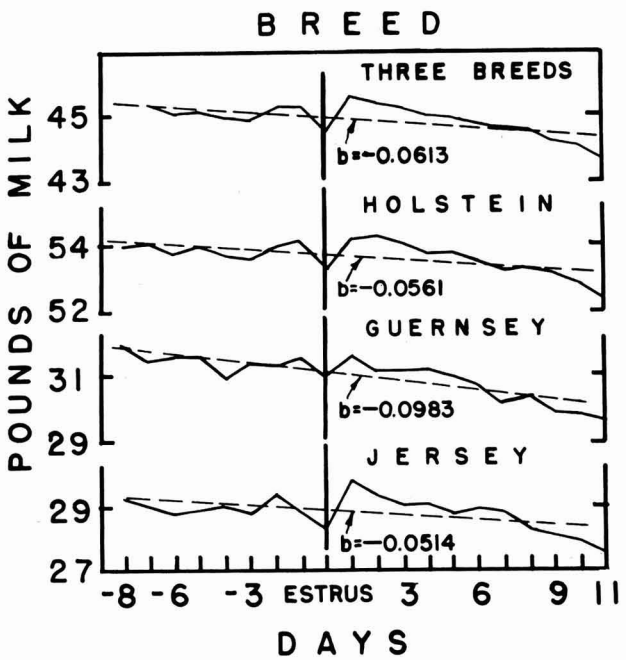

FIG. 3. Average milk yield during the estrous cycle by breeds.

more apparent as lactation progresses. Milk yield on the day of estrus exceeded that expected during the first 100 days of lactation, with decline in milk yield on the day of heat becoming progressively greater in later lactation. After 180 days in lactation, the inhibition prior to estrus is more pronounced and the increase after estrus more evident.

The greater changes in milk yield in late lactation may be related to decline in output of lactogenic hormone by the anterior pituitary. A reduction in stimulus to produce may allow greater inhibiting effects associated with endocrine

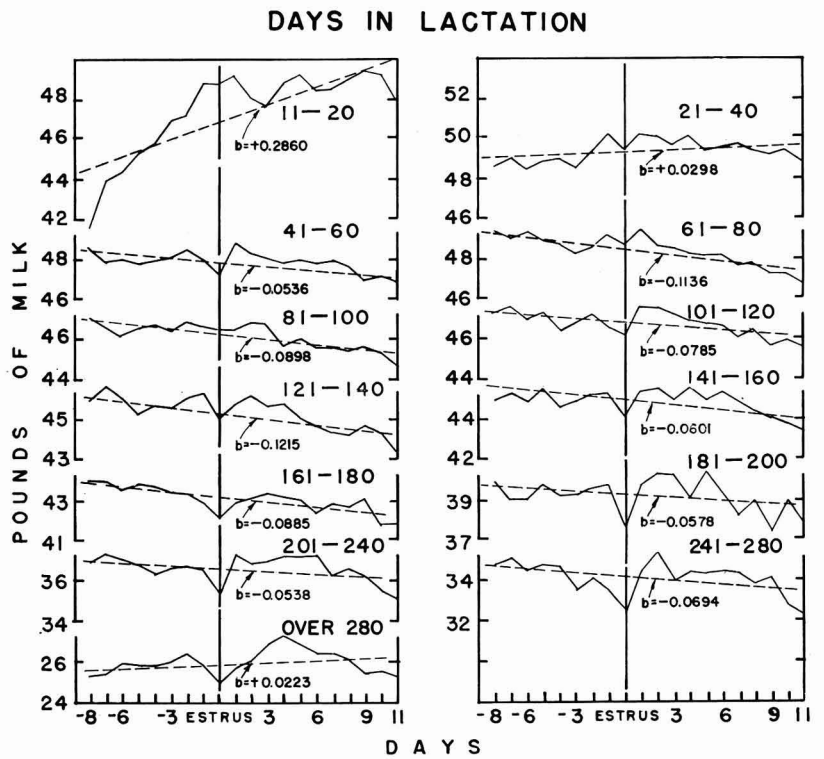

FIG. 4. Relation of stage of lactation to changes in milk yield during the estrous cycle (breeds combined).

changes of the estrous cycle. Lactogenic hormone has been shown to overcome the inhibiting effects of estrogen. Thus, such a change in endocrine balance could be a contributing factor. Cows with retained corpora lutea could conceivably gain quite an advantage in yield of milk over a lactation period as compared with a cow having regular cycles.

Variations in milk yield by breeds by times milked daily. In general, daily variation was less as determined from average decline in lactation during the 20-day period for cows milked three times daily (figure 5). This is in general agree-

ment with figure 1 with respect to variation in milk yield. However, the average fat test, F.C.M. and total fat were more variable for cows milked three times daily (fig. 1).

The variance attributable to days × times milked daily was significant. There were 140 and 499 cycles, respectively, for cows milked two and three times daily.

Variations in milk yield by months and age of cow. The variance attributable

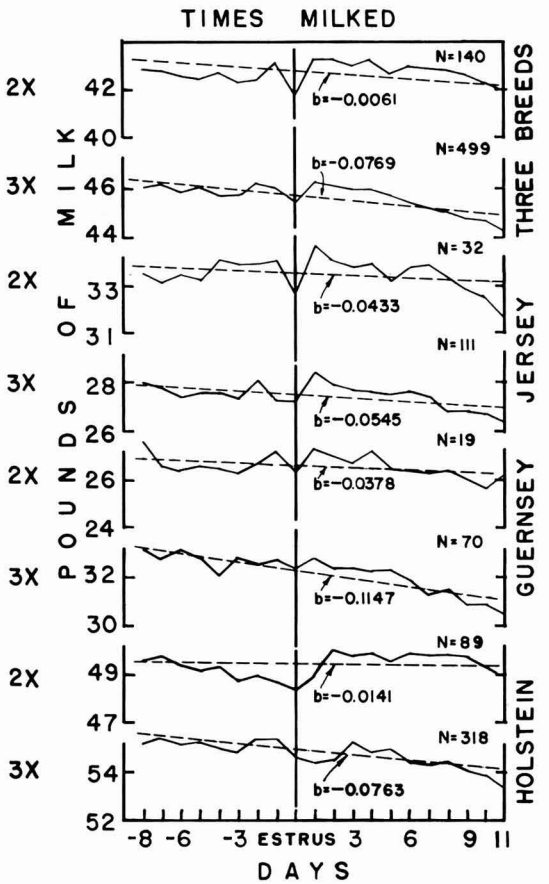

FIG. 5. Variation in milk yield by breed by times milked.

to days × months in which estrus occurred and days × age of cow approached significance. However, had the confounding effects such as times milked and stage of lactation been removed, the variance observed would have been even less. The data by months showed no consistent month-to-month pattern. Milk yield for respective days of the estrous cycle was least variable in March and December and most variable during May, June and October.

The milk yield curves by ages at which estrus occurred are shown in figure 6. The 2.0–2.5-yr. age group represents 11 Jerseys and one Holstein. This group shows more decline on the day of heat than other age groups but corresponds well with that observed for all Jerseys (fig. 2). Age of the cow appears far less important as a source of variation than stage of lactation.

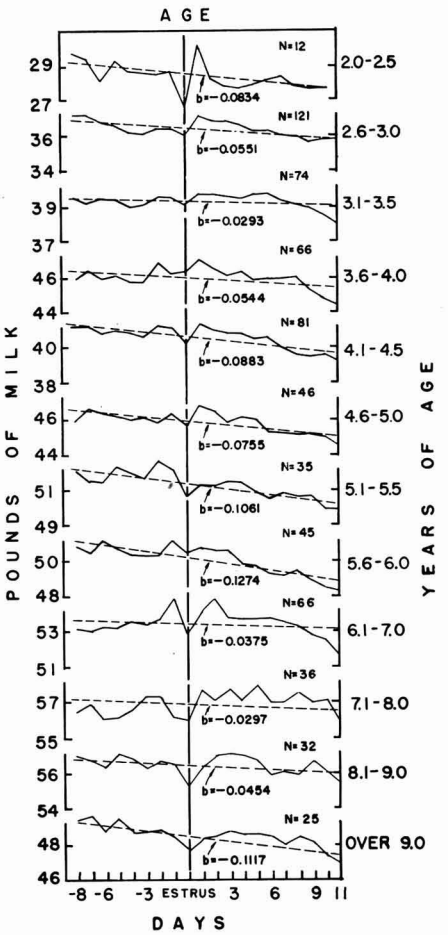

FIG. 6. Relation of age to changes in milk yield during the estrous cycle.

Variation in milk yield by days since preceding estrus. In the preceding sections, only estrous cycles longer than 18 days were used, except for the first observed cycle for each lactation. For this study, all cycles were considered. The average stage of lactation and the standard error for each classification is shown in parentheses on the right and left margins of figure 7. By virtue of their length, the longer cycles occurred later in lactation. The 67 cycles not previously

used, which were preceded or followed by estrus within 18 days, occurred at an average stage of lactation of 93 days. This is not greatly different from the

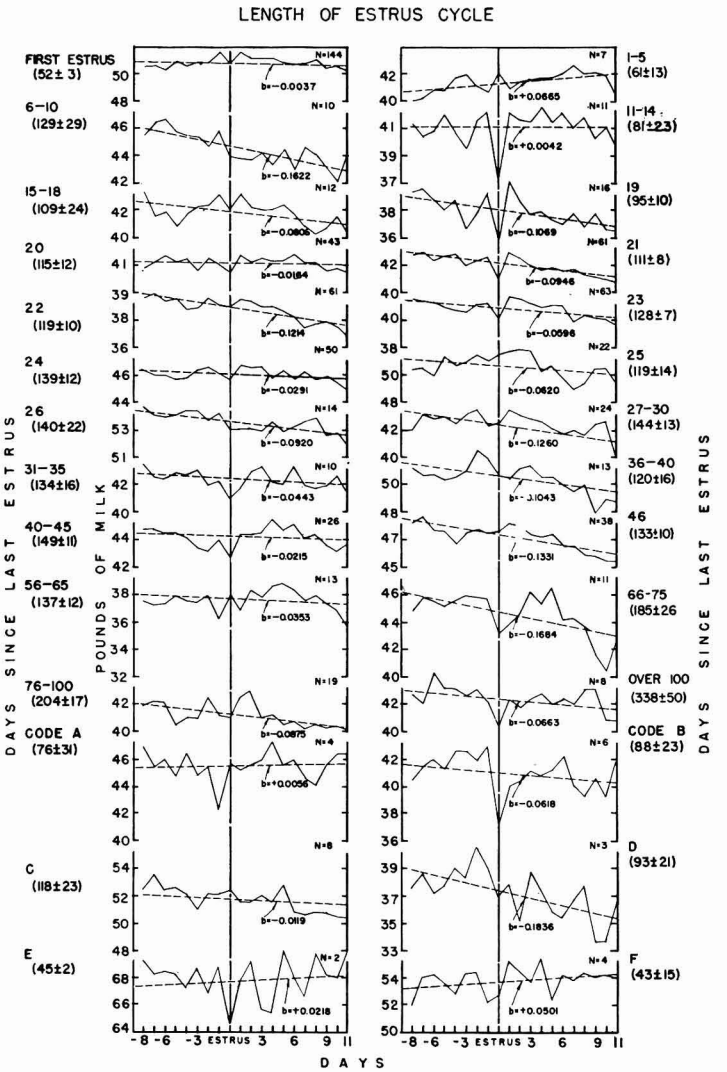

FIG. 7. Relation of length of estrous cycle to changes in milk yield.

Note: Nos. in parentheses on the margins indicate the av. stage of lactation and standard error. Codes: A = 31–32 d. cycles followed by 10–12 d. cycles; B = Estrus for 2 consecutive d.; C = 17–24 d. cycles followed by 3–8 d. cycles; D = consecutive 6–12 d. cycles; E = consecutive 4–6 d. cycles; F = First cycles followed by 3–9 d. cycles.

average of 111 days in lactation for 21-day cycles or 113 days for the 703 cycles studied.

Milk yields for each classification of estrous cycles as shown in figure 7 indicate considerable variation. The decline in milk yield on the day of heat showed considerable inconsistency throughout the range of cycles studied. No particular trend is evident. For example, cycles ranging from 11 to 14 and 19 days in length showed a marked decline on day of estrus, whereas cycles 15 to 18 days and 20 days did not show such a marked decline. There is no reason to believe this is not chance variation. The pattern of response observed for 25- and 26-day cycles likewise is inconsistent. For the most part, cycles exceeding 26 days in length do not show patterns inconsistent with those for advanced lactation (fig. 4). The lower part of figure 7 shows milk yield for irregular short cycles. Too few cases were available for each classification, but the extreme variability is rather apparent.

Seven cases of questionable heat which were available (averaged 139 days in lactation) showed the curve for milk yield to be quite characteristic as compared to cases where estrus was definitely exhibited. In addition, data were recorded for the midpoint of 42- to 46-day cycles. The midpoint was assumed the most probable date for unobserved estrus. The curve for 36 such cases (averaged 120 days in lactation) was not unlike that for observed estrus. Even though there was no pronounced dip around the time of assumed unobserved estrus, this easily could have occurred through error of assumption.

SUMMARY

Eighty estrous cycles of 19 Holstein cows were studied for variation in yield of milk, F.C.M., fat and per cent fat. All cows in estrus were kept separated from other cows in the herd. Observations were made 8 days before estrus and 11 days after. Cows milked two times daily (42 cycles) showed more variability in yield of milk but less variability in yield of F.C.M., fat and per cent fat than cows milked three times daily (38 cycles). Yield generally was higher 2 days before heat and 2 to 3 days after heat than during any other period of the cycle. Decline the day of estrus occurred when these cows were in lactation over 100 days. This decline was more apparent as stage of lactation increased. Per cent fat was higher for the 5-day period centered around estrus than any other time of the cycle, regardless of stage of lactation.

In addition to the above study, milk yield during 636 estrous cycles (over 18 days in length) was analyzed for the Guernsey, Jersey and Holstein breeds. The differences in variability of milk yield between two- and three-time milkings observed on the more limited data were confirmed. No major breed differences were observed with respect to general trend in milk yield, although the Jerseys tended to vary proportionately more.

Decline in milk yield was greatest when estrus occurred during late lactation. This decline increased as time in lactation increased. Age, month of estrus and length of estrous cycles appeared to have little consistent effect on patterns of milk yield during the estrous cycle.

This study indicates that testing cows on the day of estrus actually may be

advantageous to the cows. This particularly is true for cows in lactation less than 100 days.

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SEASONAL CHANGES IN FERTILITY OF DAIRY BULLS IN NORTHWESTERN WASHINGTON[1]

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Several studies (1, 2, 6, 7, 11, 14, 15) have yielded contradictory results relative to monthly variations in semen quality as determined by various standard laboratory procedures. Seasonal changes in breeding efficiency have been noted (4, 5, 7, 8, 9, 10, 12, 13) but the patterns of variation are in general disagreement. Anderson (1), Mercier and Salisbury (7, 8) and Swanson and Herman (14) have suggested varying environmental conditions as being responsible for the differences in experimental observations. Mercier and Salisbury (7, 8, 9) have reported an excellent series of investigations, including extensive reviews and discussions. Their results definitely point to hours of daylight as a major variable. Furthermore, it was observed that breeding efficiency of young and old dairy animals was more highly related to light than that of the medium-aged groups. High environmental temperature was not deemed a critical factor in their data, which were accumulated from eastern Canada and New York State. The evidence that high environmental temperature also may be a factor persists in the data of Erb *et al.* (4) from Indiana, Phillips *et al.* (11) from Maryland, Seath and Staples (12) from Louisiana, Hilder *et al.* (5) from Maryland and Morgan and Davis (10) from Nebraska. Variations in semen quality as judged by laboratory procedures do not necessarily coincide with observed seasonal fluctuations in breeding efficiency (1, 2, 6, 7, 14, 15).

In areas where high environmental temperatures are common during a portion of the year, those portions have shown the poorest breeding efficiency (4, 5, 10, 11, 12). Where high environmental temperatures are not common, summer and fall have shown higher average breeding efficiencies than winter and spring (7, 8, 9).

The purpose of this investigation was to note seasonal changes in semen quality and non-return rates of bulls located in northwestern Washington. This area of the United States is characteristically mild throughout the year.

EXPERIMENTAL PROCEDURE

The per cent of 60- to 90-day non-returns to first and second inseminations for all Guernsey, Jersey and Holstein bulls used for 1 or more full years by Northwest Co-op Breeders, Mt. Vernon, Washington, was tabulated for a 6-yr. period commencing August, 1944. The bulls were housed in box stalls with free access to individual exercise yards at all times. The bulls never had access to pasture and were fed silage only occasionally. Semen was used for the first 3 days following

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collection, with over half of the inseminations being made the second 24-hr. period following morning collections. Selection of semen was based largely on initial motility rating and knowledge of the previous history of the bull.

Semen quality data were gathered during a 1-yr. period starting August, 1948. Techniques employed for this study have been published elsewhere (3). Statistical analyses used in this report were according to Snedecor (13).

RESULTS AND DISCUSSION

Monthly varaitions in non-return rates. Analysis of variance of average non-return rates shown by months and years in table 1 revealed highly significant variations between both variables. The months of July through December averaged 3.4 per cent higher non-return rates than January through June. This difference was highly significant. September and October showed the highest

TABLE 1

Yearly variations in non-return rates by months for total first and second services

Months	Years						Summary[a]	Total services
	1944–45	1945–46	1946–47	1947–48	1948–49	1949–50		
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(*no.*)
August	50.4	48.6	53.0	57.2	61.9	66.3	57.2	5,284
September	57.8	55.5	59.8	64.2	58.7	63.8	61.4	3,918
October	55.8	57.7	53.7	70.1	64.7	61.0	60.9	2,447
November	58.3	48.7	53.2	63.3	64.6	64.3	60.0	4,090
December	55.1	45.5	53.6	61.2	66.4	64.2	58.4	5,854
January	55.2	45.2	42.7	54.5	64.7	60.8	55.2	7,795
February	63.7	44.9	46.3	57.8	62.4	60.5	56.5	7,908
March	48.6	43.9	46.4	55.8	65.0	61.7	55.9	9,789
April	51.5	43.7	47.5	55.9	65.0	60.5	55.5	10,284
May	52.4	45.6	51.7	55.3	65.0	64.0	57.0	12,963
June	51.4	44.8	52.6	60.3	62.1	63.4	57.5	12,096
July	52.1	49.1	57.6	57.5	65.9	66.9	59.7	10,685
Total	54.4	47.8	51.5	59.4	63.9	63.1	57.9	93,113
July–Dec.	54.9	50.8	55.2	62.2	63.7	64.4	59.6	
Jan.–June	53.8	44.7	47.9	56.6	64.2	61.8	56.2	

[a] Non-return/total 1st- and 2nd-service inseminations.

average non-return rate for the 6-yr. period, while January and April showed the lowest. There was a decided increase in non-return rate in September and October as compared with August and July for the 6-yr. period, but the data show considerable year-to-year variation. The high months of the year occurred between July and October during each of the 6 yr. It is interesting that July had the highest efficiency for 2 of the 6 yr. studied and averaged 2.7 per cent more than August. This is not easily explained except for the method of analyzing the data. The study was started in August and ended in July 6 yr. later. Any improvement in general technique during a 1-yr. period would bias the results in favor of July. The decline in August therefore is not deemed significant, and the data are interpreted as showing a steady increase in non-return rate from May through September, with essentially no difference for the months of September, October and November. These data essentially confirm the observa-

tion of Mercier and Salisbury (7, 8, 9) made under rather similar climatic conditions.

The average non-return rate for first and second inseminations, as shown in table 2, reveals little change when compared with the combined summary shown in table 1. The months of July through December averaged 3.3 and 3.6 per cent higher for first and second non-returns to service, respectively, than the months of January through June. First services averaged only 0.5 per cent more in non-return than second services. This difference is less than generally observed and could be due to selection of higher fertility bulls for second-service cows. Likewise, the number of second services recorded is higher than one would expect from the returns from first service. This is to be expected since a number of

TABLE 2

Variations in non-returns to first and second services by months

Months	1st service		2nd service	
	S.	N.R.	S.	N.R.
	(*no.*)	(%)	(*no.*)	(%)
August	3,316	57.2	1,968	57.1
September	2,423	62.4	1,495	59.8
October	1,665	60.8	782	61.3
November	2,975	59.6	1,115	60.8
December	4,378	58.3	1,476	58.7
January	5,589	55.7	2,206	54.0
February	5,500	57.1	2,408	55.1
March	6,736	55.9	3,053	55.8
April	7,239	54.7	3,045	57.3
May	9,110	57.0	3,853	57.2
June	8,216	58.5	3,880	55.4
July	7,022	60.3	3,663	58.5
Total	64,169	58.1	28,944	57.6
July–Dec.		59.8		59.4
Jan.–June		56.5		55.8

low-fertility bulls were not used for a full 12-mo. period and hence were excluded from this study.

Monthly variations in the quality of semen used for 1-yr. beginning in August, 1948. Experiments to determine the usefulness of a particular laboratory test are usually conducted for 2 to 4 mo. Variations in the value of such tests have been disturbingly frequent. Most studies of semen quality measures have shown significant month-to-month variations in many of the physical characteristics. The patterns of variation have been conflicting and do not appear to coincide with changes in breeding efficiency. The correlation of a particular semen quality measure with fertilizing capacity may then be different from one time of year to the next, even for the same population of bulls. Correlations of concentration, initial motility, motility after incubation for 30 min. at 45° C. and drop in motility during incubation with fertilizing capacity by months are shown in table 3. The average monthly values for each of these characteristics represents only semen used for insemination and hence cannot be accepted as true monthly variations in all semen produced by these bulls. Semen samples used to insemi-

nate 20 or more first- and second-service cows were used for the correlation analyses.

The correlation of each of these semen quality characteristics to non-return rate shows great month-to-month variation. Motility after 30 min. incubation at 45° C. is the only one showing a definite trend. The correlation decreases from March through July and increases from August through November, decreasing again to negative relationships in January and February.

The month-to-month variations in the correlations presented in table 3 strongly suggest further study on this problem to determine if such variations are characteristically different for different regions, different bulls and ages of bulls, and the physiological reasons, if any, for such variations. The mere existence of this type of variation makes it possible to explain differences in the various investigators' estimations of the value of a particular semen quality test

TABLE 3

Monthly variations in semen quality measurements and their correlations with fertility

Month	No. of semen samples	Initial motility		Spermatozoa concentration		Motility after 30″ incubation		Drop in motility during incubation	
		(*Rating*)	(*r*)	(*thous. /mm.*^{3})	(*r*)	(*Rating*)	(*r*)	(*Rating*)	(*r*)
August	44	8.4	0.57	1,338	0.27	5.2	0.02	3.2	0.37
September	27	8.3	−0.12	1,324	−0.15	5.2	0.15	3.0	−0.34
October	28	8.4	0.66	1,417	−0.27	6.0	0.21	2.4	−0.07
November	46	8.5	0.27	1,442	0.22	5.6	0.33	2.8	−0.05
December	52	8.8	−0.25	1,366	−0.30	5.6	−0.01	3.1	−0.19
January	57	8.6	0.09	1,439	−0.04	5.3	−0.13	3.3	−0.04
February	50	9.0	−0.22	1,617	−0.14	5.7	−0.21	3.3	0.07
March	53	8.5	0.18	1,372	0.00	6.2	0.22	2.3	0.06
April	61	8.2	0.25	1,521	−0.24	5.6	0.56	2.6	−0.12
May	58	8.1	0.23	1,226	0.24	5.9	0.13	2.2	0.12
June	58	8.6	0.11	1,376	0.30	6.1	0.02	2.5	0.08
July	57	8.1	0.19	1,368	0.16	6.1	0.01	2.0	0.16
Total	591	8.4	0.14	1,375	0.03	5.7	0.02	2.7	0.01

for predicting fertilizing capacity. Hence, it does not appear possible to estimate accurately the true value of a semen quality test when experimental periods of less than 1 yr. are used.

SUMMARY

The per cent 60- to 90-day non-returns to 93,113 first- and second-service inseminations for Guernsey, Jersey and Holstein bulls used for artificial breeding purposes in northwestern Washington over a 6-yr. period were tabulated for this study. Monthly variation was highly significant, with January showing the lowest and September the highest average non-return rates. Non-return rate was lowest during January, February, March and April and gradually increased to the highest level in September, October and November. This pattern of breeding efficiency essentially parallels the results Mercier and Salisbury (7, 8, 9) observed from data collected in New York and eastern Canada.

Monthly correlations of sperm concentration, initial motility, motility after 30-min. incubation at 45° C., and drop in motility during incubation with non-return rates revealed great variation in the relationships from one period of the year to the next. Only 591 semen samples collected during 1 yr. were involved, but the data strongly suggest that the value of semen quality measurements cannot be accurately estimated during experimental periods of less than 1 yr.

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THE BULL AS ONE CAUSE OF DELAYED RETURNS TO SERVICE IN ARTIFICIAL BREEDING

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In an earlier report (11) age of extended semen was implicated as contributing to the magnitude of the difference between 1-mo. and 5-mo. non-returns to service. While it is not known that pregnancy actually was initiated in the cows involved, from the careful work of Tanabe and Casida (12) with cows of low fertility it may be inferred that such was the case. Consequently, these delayed returns to service are considered as indirect evidence for early embryonic mortality. It is the purpose of this report to give the results of studies designed to determine the extent to which the bull may be responsible for delayed returns to service in artificial breeding and, by inference, a contributor to embryonic mortality.

DATA AND STATISTICAL ANALYSES

Using the data from the insemination records of the New York Artificial Breeders' Cooperative, Inc., statistical studies were made of the 1- and 5-mo. non-return percentages and the difference, delayed returns, between these two figures for a large number of bulls. Since the basic data contained time trends reflecting the expansion of an artificial breeding organization, it was necessary to select carefully the data to be studied in order that these trends and other biases, such as the number of services contributing to the percentages (4), might be reduced to a minimum.

The first study consisted of data from three Holstein and three Guernsey bulls used each month during 1943 by 12 technicians to inseminate 6,056 cows. The six bulls differed significantly in their fertility levels, ranging from 73 to 55 per cent for the 1-mo. non-returns and from 59 to 37 per cent for the 5-mo. non-returns. The difference between bulls in delayed returns varied significantly, the bull means ranging from 11 to 18 percentage units, with an over-all mean of 15 percentage units.

The correlation coefficient between 5-mo. non-returns and the difference between 1-mo. and 5-mo. non-returns was negative, -0.81, and significant at the 5 per cent level of probability. The same calculation between 1-mo. non-returns and the difference resulted in a correlation coefficient of -0.69. This correlation means that high 1-mo. non-return rates were associated with small differences between 1-mo. and 5-mo. non-return rates. Similar calculations for inseminator-technicians and for seasons of the year revealed no significant differences be-

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tween non-return rates and apparent embryonic mortality. Thus, it appears that in this study the bulls contributed the major fraction of the observed variability in embryonic mortality as measured by delayed returns.

A second study was made of the 1-mo. and 5-mo. non-returns for 29 Holstein, Guernsey, Jersey and Ayrshire bulls used during 12 consecutive months (March, 1946–February, 1947) to inseminate 61,265 first-service cows.

Again the primary effect on delayed returns was contributed by the bulls, as is evidenced by the negative correlation shown in table 1. These correlation coefficients between bulls are remarkably similar to those found in the first analysis for the six individual bulls. They imply that nearly one-half of the variability among bulls in the failure of groups of cows to maintain pregnancy during the 1- to 5-mo. interval after the month of insemination was associated with the differences in original fertility levels of the bull. The difference in delayed returns by bulls ranged from 9 percentage units for the high-fertility bulls to 16 percentage units for the low-fertility bulls.

TABLE 1

Correlation coefficients between delayed returns and 1-mo. non-returns and 5-mo. non-returns to service

	df	r between delayed returns and:	
		1-mo. non-returns	5-mo. non-returns
Among breeds	3	−0.02	−0.40
Among bulls within breeds	25	−0.69**	−0.84**

** $P < 0.01$.

To test further these observations and to test specifically the question of the effect of the varying numbers of first-service cows on the validity of the percentage estimates of embryonic mortality as measured by delayed returns for each bull for each month, a third study was made using the available records prior to November, 1947, on the basis of the period of maximum length during which the largest number of bulls was used for insemination each month. During the 18-mo. period from March, 1946, through August, 1947, 22 bulls had been used each month to inseminate 79,370 first-service cows. The number of cows inseminated with the extended semen of an individual bull in any 1 mo. varied from a minimum of four (two cases less than 10 and 19 cases less than 30) to a maximum of 837 (20 cases over 500). Analysis of the variability of delayed return percentages attributable to the varying numbers of services on which the percentages were calculated, as outlined by Cochran (4), indicated that somewhat more than one-half (57 per cent) of the error mean square was binomial variance. Therefore, partial weighting was used in the analysis of variance to test the effect of bulls and months upon the difference between 1- and 5-mo. non-return percentages. The method of partial weighting selected involved weighting all percentage figures based on less than 25 cows by the actual number of cows inseminated. All percentage figures based on more than 25 cows were given equal weight. In addition, an analysis of variance of the unweighted percentages was

TABLE 2

Mean squares calculated for bulls and months by two different methods of analysis of percentages of delayed returns

	df	Partial weighting	Equal weighting
Bulls	21	3,124**	65.2**
Months	17	2,601**	59.3**
Error mean square	357	823	19.2

** $P < 0.01$.

calculated for comparative purposes. As shown in table 2, both methods of analysis gave similar bull and month mean squares in proportion to the error mean squares, from which it was concluded that the use of unweighted percentages in the preliminary studies was justified.

These analyses show that a real difference existed between bulls in the proportion of the cows which showed delayed returns to estrus after insemination, and thus presumably bulls were responsible for much of the variation found in the incidence of embryonic mortality. The means for bulls ranged from 8 to 16 percentage units with an over-all mean of 11.9 ± 0.24 (S.E.) percentage units. The differences among months in delayed returns were highly significant statistically, the largest proportion occurring in January, February and March, and the lowest in May and June of each year. Also, analyses of variance of the 1-mo. non-returns and the 5-mo. non-returns showed highly significant differences in the non-return rate of bulls. However, among months the differences were small and non-significant for the 1-mo. non-returns, but were larger and highly significantly different for the 5-mo. non-returns.

The correlation coefficients calculated between the 1- and 5-mo. non-returns and between each of these measures of fertility and the level of delayed returns to service among bulls and among the months in which the insemination occurred are shown in table 3. Here again highly significant negative correlations existed between the measures of fertility and the apparent embryonic mortality rate among bulls. The values are only slightly lower in each instance than in the previous less carefully controlled analysis. From these results the conclusions are inescapable that bulls are a major source of origin of the phenomenon observed and that whatever factor or factors are responsible for the inability of fertilized ova to implant and for pregnancy to be maintained, as evidenced by

TABLE 3

Correlation coefficients between 1-mo. non-returns (X), 5-mo. non-returns (Y) and the differences between the two measures of fertility level, delayed returns (Z)

	df_r	r_{XY}	r_{XZ}	r_{YZ}
Among bulls	21	0.96**	−0.56**	−0.78**
Among months	17	0.24	0.71**	0.00
Error	354	0.84**	0.00	−0.32**

** $P < 0.01$.

late returns to estrus after insemination, also are operative in establishing a low original level of conception as measured by 1-mo. non-returns. Also, it appears that the incidence of embryonic mortality may be associated with the months and seasons of the year.

DISCUSSION

Three items of significance in determining the per cent of cows which return late to service after artificial insemination have been established by this and the earlier investigations, *viz.,* (a) the bull as the source of the semen used for the inseminations, (b) the age of the extended semen used and (c) seasonal effects, as measured by month-to-month variations.

There are, no doubt, numerous possible causes of these delayed returns to service. They may have been caused by the presence in the semen of pathogens of yet unincriminated types such as *Vibrio foetus,* since all bulls in these studies are free, so far as routine veterinary tests and examinations would discern, from tuberculosis, brucellosis and trichomoniasis. Unknown pathogenic organisms, if the cause, would logically be contributed in different degrees by the different bulls. Most of the inseminations in these studies were made before the now common antibiotics and chemotherapeutic agents were added to the extended semen, although sulfanilamide was used routinely after January 6, 1947. Some of these agents have been found to improve fertility levels when used in the semen of some low-fertility bulls (1, 2, 3, 5, 7). No careful studies of the effects of these agents on embryonic mortality have been reported to date. The results of Salisbury and Bratton (10) and of Rottensten (8, 9) suggest that sulfanilamide additions may reduce embryonic mortality, although the recent evidence of Almquist (2) does not support that point of view. The report of Easterbrooks *et al.* (5) of an inverse relationship between the response in increased fertility level by additions of streptomycin to semen and the initial fertility level of bulls suggests that antibiotics are effective in reducing embryonic mortality.

A problem of this character might be aggravated by the common practice of intra-uterine insemination resulting in the virtual elimination of the cervix as a barrier to bacterial invasion of the uterus.

End-products of spermatozoan metabolism resulting from handling and storage of semen may be at the foundations of the problem, since at least one of the possible end-products, H_2O_2 (13), is known to produce mutations in bacteria (16) and to retard cleavage of arbacia eggs fertilized by spermatozoa exposed to H_2O_2 (6). VanDemark *et al.* (14) have shown that H_2O_2 reduces spermatozoan motility, though in further studies (15) the addition of catalase to yolk-citrate extenders had no effect on fertility.

Finally, the seasonal effect observed may be due to the insemination of cows in estrus just prior to the occurrence of a seasonal anestrus, resulting in considering them pregnant to insemination, but upon resumption of estrous cycles with increasing length of daylight becomes numbered amongst the delayed returns, an event unassociated with the actual insemination.

SUMMARY

The results or statistical studies indicate that the semen of bulls is one of the major sources of origin of the significant differences observed from bull to bull in the incidence of embryonic mortality, as measured by the difference between 1- and 5-mo. non-returns to service in artificial breeding. The level of embryonic mortality was negatively and highly significantly correlated with the original fertility level of bulls as measured by 1-mo. non-returns, the correlation coefficients being in the neighborhood of -0.6 to -0.7. A significant effect of the season of the year on apparent embryonic mortality was indicated, the maximum effect being produced from inseminations in January, February and March, the minimum being in May and June.

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THE EFFECT OF TIME AND OTHER FACTORS ON THE NON-RETURN TO SERVICE ESTIMATE OF FERTILITY LEVEL IN ARTIFICIAL INSEMINATION OF CATTLE

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For the successful operation of an artificial breeding unit, reliable estimates of fertility are essential. The dairyman is primarily interested in the number of services per live calf born. As a practical estimate of the efficiency of the technique of artificial insemination, this measure of fertility is impossible to obtain for a large population. Therefore, quicker and easier methods are required. Manual pregnancy diagnosis and the non-return to service estimate are the two most logical methods available at the present time. While pregnancy diagnosis might appear to be the more useful estimate of fertility, in reality it contains most of the errors inherent in any estimate of fertility made before calving and is more difficult to obtain routinely.

Most artificial breeding organizations have come to rely on a calculation from the insemination records to arrive at estimates of the efficiency of their operations. The method, universally known as the non-returns to service estimate of fertility, has been described earlier (8). Most research workers have been forced by circumstances to adopt the same method for determining the effect of varying semen treatments or other factors on fertility in artificial insemination.

Among the obvious drawbacks of the method are that some cows fail to conceive from insemination and may be disposed of before the next estrus, some may be bred naturally at the next estrus, while in others estrus may not be detected. All would be considered as pregnant and would make the estimate of the conception rate higher than the true figure. Casida *et al.* (3) and Barrett *et al.* (1) found that 4 mo. after service the non-return to service calculation overestimated the level of conception actually found by pregnancy diagnoses made from 35 to 49 days after insemination.

Elliott (5) checked 219 cows directly on farms and found that 68 per cent had not returned to estrus in 1 full month following the month in which the cows were inseminated and 53 per cent had not returned within 5 mo. Of these 219 cows, only 49 per cent were diagnosed as pregnant or produced a living calf, which is 19 percentage units less than the 1-mo. non-returns and 4 percentage units less than the 5-mo. non-returns. However, all biases in non-return calculations do not result in overestimation of conception. Studies (2, 4) indicate that about 3.5 per cent of pregnant cows exhibit typical signs of estrus. Such cows might be reinseminated and thus might not be recorded as pregnant from

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the earlier insemination. In other cows fertilization may occur, but embryonic mortality may supervene (11). The effect of vibriosis and brucellosis as causes of fetal mortality is well known and obviously contributes to the error of fertility estimates made at an earlier date by either method.

If non-returns to service data are to be used, the major factors contributing to their error must be known and the magnitude of the error established. It is the purpose of this paper to present evidence on some factors affecting the non-return to service estimate of fertility level.

DATA AND DISCUSSION

The data presented herein were taken from the records of the New York Artificial Breeders' Cooperative, Inc., Ithaca, N. Y., during the period from January 1, 1943, to August 1, 1947, and consisted of information on the individual cows inseminated or reinseminated within 5 mo. after the month of insemination. In all cases the semen used was extended with either citrate-yolk or citrate-sulfanilamide-yolk extender and shipped to member units where inseminations were made up to 7 days after collection with approximately 1 ml. of extended semen being deposited in the uterus.

The returns and non-returns to service were summated at 1 full month and at 5 full months after the month in which the cows were inseminated. In other instances the summation was made after 1, 2 and 5 mo. and after 1, 2, 3, 4 and 5 mo. In general, the figure of primary concern was the difference between 1-mo. and 5-mo. non-returns. This value cannot be taken as strictly representing the difference between original conceptions and final holding to service, for in the work of Tanabe and Casida (11) the considerable loss of original fertilizations was earlier in pregnancy than the 45-day average interval from insemination to the date of calculation of original fertility level. It is believed that what is measured here as the difference between 1-mo. and 5-mo. non-returns is due in a large part to failure of implantation or embryonic mortality resulting in the delay, for a variable time interval, of the return to estrus.

Relation of 1-mo. non-returns to 5-mo. non-returns to service. The relationship of 1-mo. to 5-mo. non-returns for slightly more than 225,000 cows is presented in table 1. From these data it is apparent that the magnitude of the difference between 1-mo. and 5-mo. non-returns was smaller as experience with the artificial breeding program advanced. Whether or not this latter change was caused by a decrease in the incidence of disease in the herds involved or by a change in technique of one type or another, including more careful semen handling, is not shown by the data at hand.

The effect of the order number of insemination. On the basis of 25,305 first-service and 12,301 second-service cows, the 1- and 5-mo. non-return percentages were 66.6 and 51.7, respectively, for the first-service cows, and 63.4 and 48.5, respectively, for the second-service cows. The decrease between the 1- and 5-mo. non-returns was 14.9 percentage units for both groups of cows.

Breed and seasonal differences. There was a significant breed difference in the decrease from 1- to 5-mo. non-return percentages, the decrease being greatest

TABLE 1

Effect of time after insemination on fertility level estimates based on non-returns to first service and the difference between 1-mo. and 5-mo. non-returns

Date	Jan., '43–Dec., '44	Aug., '45–July, '46	Aug., '46–July, '47	4 experiments[a]
No. first-service cows	25,305	73,327	118,950	14,263
1-mo. non-returns (%)	66.6	67.6	70.5	69.0
2-mo. non-returns (%)	.....	59.9	62.1	61.2
3-mo. non-returns (%)	.....	.....	.....	58.5
4-mo. non-returns (%)	.....	.....	.....	57.1
5-mo. non-returns (%)	51.7	55.8	57.6	56.4
Difference between 1-mo. and 5-mo. non-returns (%)	14.9	11.8	12.9	12.6

[a] Four experiments testing other questions conducted from 1942 through 1946.

for the breed with the lowest original fertility. However, this difference may have been a consequence of the sample of bulls involved.

The 1- to 5-mo. differences for months were significant ($P < 0.05$), particularly for November in which the decrease each year was greater than normal. However, since the monthly changes did not shift gradually with the seasons, the importance of this finding is obscure.

The effect of the age of the extended semen. To study the effect of the age of the extended semen on its fertility and on the decrease in 1- to 5-mo. non-returns for each day, a series of semen samples was selected, all of which had been used by the same inseminator-technicians on the fourth day after collection. By this method of selecting the data it was believed possible to obviate the bias caused by the inseminators' routine day-to-day selection of semen samples for use.

The results are shown in table 2 and indicate, by the highly significant regression between the day of use and the difference between 1- and 5-mo. non-return percentages, that the age of the semen plays some part in determining the magnitude of delayed returns.

Other possible causes of delayed returns. No satisfactory data are available for determining whether or not delayed return to service is a phenomenon found in artificial insemination and not found in natural service. The cow effect on the incidence of delayed returns is not subject to direct testing in the data at hand, though it is reasonable to assume that variability among cows is involved.

TABLE 2

The effect of the length of time of storage of extended semen on its fertility level and the difference between 1-mo. and 5-mo. non-returns

	Age of extended semen when inseminated in relation to day of collection				
	Same d.	2nd d.	3rd d.	4th d.	5th d.[a]
No. of inseminations	12	726	756	970	56
1-mo. non-returns (%)	58.3	67.0	62.8	54.3	57.2
5-mo. non-returns (%)	50.0	57.0	50.7	41.5	39.3
Difference (%)	8.3	10.0	12.1	12.8	17.9

[a] 5th d. or more.

Intra-uterine insemination, routinely practiced in the operation from which the present data were obtained, has been criticized as a possible cause of uterine trauma which might contribute to the early interruption of pregnancy (10).

Nutritional deficiencies at the end of the barn-feeding period do not appear to be causes of delayed returns in view of the fact that in the data studied here the month of November (the end of the pasture season) during 2 consecutive years had the greatest decrease between 1- and 5-mo. non-returns.

Salisbury and Bratton (9) have reported a trend towards a greater difference between 1-mo. and 5-mo. non-returns for semen extended at rates of 1:400 or more as compared to lower rates. The addition of sulfanilamide tended to reduce, but did not eliminate, this difference. Also, Rottensten (6, 7) has reported that the addition of sulfanilamide to the extender reduced the incidence of delayed returns.

SUMMARY

Evidence is presented indicating that in artificial insemination of cattle a significant proportion, varying up to 25 per cent, of the cows thought to be pregnant after one insemination, because they failed to show estrus during a time interval after insemination equivalent to two normal estrous cycles, actually returned for service at a later date. The difference between the percentage of cows that had not returned for reinsemination during 1 full month after the month of insemination (1-mo. non-returns) and those that did not return to service during the 4 subsequent months (5-mo. non-returns) was of the order of 12 to 15 percentage units. The magnitude of the difference varied with the age of the extended semen used for insemination and with experience in the artificial breeding program.

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The authors gratefully acknowledge the assistance of the management and the records section of the New York Artificial Breeders' Cooperative, Inc., for their aid in making available the records of services and returns to service for this study and the assistance of Mrs. Elizabeth Dunn and Mrs. Lois DeChellis in the statistical analyses of the data.

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THE INFLUENCE OF ANTIBIOTICS ON DELAYED RETURNS IN ARTIFICIAL BREEDING

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The problem of delayed returns to services in artificial breeding and its incidence in relation to the fertility level of bulls has been discussed by Salisbury *et al.* (9).

Since antibiotics have been shown to affect the fertility of bulls used in artificial breeding (1, 2, 3, 4, 5, 6), the question arises as to the effects of these agents upon delayed returns.

DATA AND DISCUSSION

Based on experimental evidence previously reported (6), the New York Artificial Breeders' Cooperative, Inc., on August 4, 1949, began adding routinely 500 units of penicillin and 500 units of streptomycin to each milliliter of the standard 2.9 per cent citrate-sulfanilamide-yolk extender then in use. Data presented herein are from a study of the returns and the non-returns to service during a period of 8 mo. before and 17 mo. after the Cooperative made the change to the routine use of antibiotics. The data for the five dairy breeds, Holsteins, Guernseys, Jerseys, Ayrshires and Brown Swiss, have been combined, since a study by breeds indicated that each behaved similarly with respect to the criteria studied.

Table 1 shows the monthly average 28- to 35-day, the 60- to 90-day and the 150- and 180-day non-returns for 345,666 first-service cows and the differences between these three estimates of conception rate (delayed returns) during the periods before and after the change to the routine use of the antibiotics. The points of primary interest in these data are the immediate and sustained increase in the percentage of non-returns and the precipitous decrease in the percentage of delayed returns after August 4, 1949. The difference between the 28- to 35-day and 60- to 90-day non-returns was 15.0 percentage units when antibiotics were not used and 9.5 percentage units when they were used. In contrast, the difference between the 60- to 90-day and the 150- to 180-day non-returns was 3.8 percentage units before and 3.3 percentage units after the change. A similar pattern of delayed returns was found to exist for 95,570 second-service cows and 38,511 third-service cows.

The smaller percentage of delayed returns (28- to 35-day minus 60- to 90-day) accompanying the routine use of penicillin and streptomycin is interpreted as evidence that the percentage of embryonic deaths was decreased through the inhibition of infectious organisms by this combination of antibiotics. Additional support for this interpretation of the sudden decrease in delayed returns accompanying the routine use of antibiotics was obtained in a carefully controlled experiment (6, 7) in which time was not a confounding factor.

Table 2 shows the non-returns and delayed returns for 36 bulls used exten-

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sively each month for a period of 8 mo. before and 8 mo. after the change to the routine use of antibiotics. Of particular interest in this table is the among-bull variation before and after the change. For the delayed returns the "before" variance ($\hat{\sigma}^2$) was approximately ten times greater than the "after" variance. The within-bull variance essentially was unchanged. Only a part of the decrease in among-bull variance was expected on the basis of the 30 per cent

TABLE 1

Non-returns to 1st services for all breeds by months before and after the change to the routine use of antibiotics in the semen extender

Month	No. of 1st services	Per cent non-returns to service			Differences		
		28- to 35-d.	60- to 90-d.	150- to 180-d.	28- to 35-d. minus 60- to 90-d.	60- to 90-d. minus 150- to 180-d.	28- to 35-d. minus 150- to 180-d.
Dec. '48	18,689	74	60	56	14	4	18
Jan. '49	16,798	74	60	55	14	5	19
Feb. '49	12,863	78	63	59	15	4	19
March '49	12,770	80	64	60	16	4	20
April '49	11,821	81	67	64	14	3	17
May '49	14,056	78	65	62	13	3	16
June '49	14,687	82	69	66	13	3	16
July '49	10,628	82	67	63	15	4	19
Total	112,312						
Weighted mean		79.1	64.1	60.3	15.0	3.8	18.8
	Changed to extender containing penicillin and streptomycin						
Aug. '49	8,254	84	73	70	11	3	14
Sept. '49	6,329	84	74	70	10	4	14
Oct. '49	6,685	85	76	72	9	4	13
Nov. '49	12,256	84	74	71	10	3	13
Dec. '49	20,398	83	74	71	9	3	12
Jan. '50	17,381	83	73	69	10	4	14
Feb. '50	13,229	83	72	68	11	4	15
Mar. '50	14,879	83	73	70	10	3	13
April '50	14,730	83	73	70	10	3	13
May '50	16,561	81	72	70	9	2	11
June '50	18,620	81	73	70	8	3	11
July '50	13,548	83	74	71	9	3	12
Aug. '50	10,475	84	75	71	9	4	13
Sept. '50	7,787	84	75	71	9	4	13
Oct. '50	8,466	85	76	72	9	4	13
Nov. '50	15,659	82	71	67	11	4	15
Dec. '50	28,097	80	71	67	9	4	13
Total	233,354						
Weighted mean		82.5	73.0	69.7	9.5	3.3	12.8

decrease in the mean level of delayed returns to service resulting from the use of the two antibiotics. Analysis of variance further revealed that bulls differed significantly in the percentage of delayed returns before antibiotics were used ($P < 0.01$), but did not differ in this respect during the period when antibiotics were used.

As a consequence of the large decrease in the variance for delayed returns among bulls, accompanying the use of antibiotics, the correlations between the non-return rates at different time intervals following service were increased, as

TABLE 2

Non-returns to 1st service by bulls before and after the change to the routine use of antibiotics in the semen extender[a]

Bull no.	Before		After		Bull no.	Before		After	
	28- to 35-d. % N.R.	Delayed returns[b]	28- to 35-d. % N.R.	Delayed returns		28- to 35-d. % N.R.	Delayed returns	28- to 35-d. % N.R.	Delayed returns
1	74.4	20.5	79.8	10.5	19	80.1	11.5	81.0	11.5
2	84.8	11.1	85.3	8.8	20	80.0	13.2	84.4	9.6
3	84.4	9.8	84.8	9.5	21	79.9	12.1	83.9	8.8
4	75.8	14.2	84.5	8.8	22	79.4	9.3	81.6	9.8
5	81.7	13.1	85.5	8.5	23	76.6	18.3	83.6	9.7
6	76.6	14.7	78.5	10.7	24	80.9	14.2	82.4	11.0
7	78.2	17.3	85.1	7.6	25	76.0	17.4	79.5	10.6
8	68.8	15.3	72.3	13.2	26	80.3	9.1	85.2	11.4
9	80.6	17.1	84.1	10.0	27	77.6	10.9	78.7	9.7
10	79.7	17.8	85.0	9.3	28	77.4	16.1	83.8	10.4
11	81.9	19.3	87.2	10.2	29	81.7	12.1	82.1	9.7
12	83.1	12.3	83.3	8.7	30	73.4	17.1	79.9	11.2
13	80.8	15.2	85.6	9.8	31	77.5	10.2	78.5	9.4
14	75.9	18.1	82.2	10.7	32	82.0	20.2	88.4	9.2
15	80.2	20.0	84.5	10.7	33	76.4	18.2	83.6	6.7
16	83.2	9.3	85.3	9.8	34	78.2	10.5	86.5	10.3
17	76.8	13.6	79.9	10.2	35	79.3	10.4	83.4	10.3
18	81.1	18.8	81.5	9.9	36	83.5	8.0	85.5	9.6
Mean (unweighted)						79.1	14.3	83.0	9.9
Variance ($\hat{\sigma}^2$)							13.67		1.32
Standard deviation ($\hat{\sigma}$)							3.70		1.15

[a] There were 77,834 first services before the change and 57,944 first services after the change to the routine use of antibiotics in the semen extender.

[b] The delayed returns in this table are defined as the difference between the 28- to 35-day and the 60- to 90-day per cent non-returns to 1st-service cows.

shown in table 3. From the correlation coefficients in this table it is obvious that the average 60- to 90-day and 150- to 180-day per cent non-returns for a bull now can be accurately predicted from the cows not returning to service within 28- to 35-days following service. The differences between the "before" correlation coefficients of 0.80 and 0.75 and the "after" correlation coefficients of 0.97 were significant at the 1 per cent level of probability.

In contrast to the increases in the among-bulls correlations, the within-bulls correlations were essentially the same before and after the beginning of the routine use of antibiotics. The within-bulls correlations between the 28- to 35-

TABLE 3

Linear correlations between the per cent non-returns at different intervals following service

Interval between returns to service	Among-bulls linear correlation coefficients	
	Before antibiotics	After antibiotics
Between 28- to 35-d. and 60- to 90-d. % non-returns	0.80[a] (0.57 to 0.91)[b]	0.97[a] (0.93 to 0.99)
Between 28- to 35-d. and 150- to 180-d. % non-returns	0.75[a] (0.48 to 0.89)	0.97[a] (0.93 to 0.99)
Between 60- to 90-d. and 150- to 180-d. % non-returns	0.99[a] (0.97 to 0.996)	0.99[a] (0.97 to 0.996)

[a] $P < 0.01$.

[b] 1% confidence limits.

day and the 150- to 180-day per cent non-returns were 0.74 before and 0.75 after the change to the use of antibiotics. These correlations are significant at the 1 per cent level of probability, but the small non-significant difference between them indicates that antibiotics had similar effects on semen samples from a particular bull.

It appears that the principal effect of the antibiotics was to decrease the delayed returns occurring between 28- to 35-days and 60- to 90-days after service by those bulls which previously had been low in fertility. There was little effect on the high fertility bulls. While the remaining percentage of delayed returns tended to be equalized for all bulls sampled, the specific bull effect was not entirely eliminated by the antibiotics used. The report by Salisbury *et al.* (9) showing that a high negative correlation (– 0.7) existed between the 30- to 60-day per cent non-returns and the per cent delayed returns is supported by the data in this study. The among-bulls correlations between per cent non-returns and delayed returns (28- to 35-day per cent non-returns minus 60- to 90-day per cent non-returns) were – 0.36[a] before and – 0.57[b] after the change to the use of antibiotics. Similar values were obtained for the relationship between the 28- to 35-day per cent non-returns and the delayed returns occurring up to 150- to 180-days.

These negative correlations indicate that the bulls with the highest 28- to 35-day non-return rates also tended to have the fewest delayed returns. This appears to be a biological phenomenon because, on the basis of chance alone, one would expect to obtain a positive correlation. The higher 28- to 35-day percentage figures would be expected to decrease more by 60- to 90-days or 150- to 180-days than would the lower 28- to 35-day figures (8). Consequently, the true significance of these correlations is greater than one obtains by testing them against an expected correlation coefficient of zero. Since these correlations reflect a real biological phenomenon, it is reasonable to assume that the semen *per se,* as suggested by Salisbury *et al.* (9), and particularly infectious agents controllable by penicillin plus streptomycin, as indicated by the data in this report, are important origins of the differences among bulls in embryonic mortalities, as measured by delayed returns to service in artificial breeding.

The residual delayed returns, characteristic of bulls in general, have several possible causes. They may result from some ova being fertilized by spermatozoa inherently incapable of maintaining embryonic development, or by spermatozoa which have become defective during the semen handling and insemination procedures common to all bulls used within an organization. Also, these returns may reflect ovum or intra-uterine failures characteristic of the population of cows inseminated.

SUMMARY

A study of 112,312 first-service cows bred artificially during an 8-mo. period immediately prior to the routine addition of 500 units each of penicillin and streptomycin per milliliter of citrate-sulfanilamide-yolk extender revealed that

[a] $= 0.01 < P < 0.05$ when tested against an expectancy of $r = 0$.

[b] $= P < 0.01$ when tested against an expectancy of $r = 0$.

the 28- to 35-day, 60- to 90-day and 150- to 180-day per cent non-returns were 79.1, 64.1 and 60.3, respectively. The corresponding values for 233,354 first-service cows bred during a 17-mo. period immediately following the change were 82.5, 73.0 and 69.7. The smaller percentage of delayed returns when antibiotics were used is interpreted as indirect evidence for a marked decrease in embryonic mortalities associated with control of infectious agents in semen.

Bulls' fertility (28- to 35-day per cent non-returns) before as well as after the use of antibiotics was negatively correlated with the percentage of delayed returns, indicating that the semen *per se* also is a source of origin of some of these delayed returns.

The among-bull variance for non-returns to first-service cows was reduced to less than 10 per cent of its original value by the addition of the antibiotics, penicillin and streptomycin, to the citrate-sulfanilamide-yolk extender.

When antibiotics were used, the 150- to 180-day non-return percentages were predicted nearly as accurately from the 28- to 35-day non-returns as from the 60- to 90-day non-returns.

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THE EFFECT OF DOSAGE LEVEL AND VARIOUS METHODS OF ADMINISTRATION ON THE CONCENTRATION OF DDT IN MILK[1]

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Several investigators (2, 3, 4, 5, 11, 14, 15, 16, 17) have reported appearance of DDT in the milk of dairy cows following consumption of forages sprayed with DDT. On the other hand, some experiments have been reported (1, 5, 7) in which cows have been fed forage previously sprayed with DDT and little or no DDT could be detected in the milk. It is difficult to compare the results of various workers reporting the concentration of DDT in bovine milk when using various forages, different levels of spraying, different spray preparations, various levels of milk production and different methods of analysis.

The object of this study was to investigate some of the factors that might affect the absorption of DDT and its rate of excretion into the milk of dairy cattle.

EXPERIMENTAL PROCEDURE

The animals selected for this study were normal, healthy, milking cows as well as could be ascertained by records of their previous health history and examination of the animals by a veterinarian. They were fed alfalfa hay and a mixture of 100 parts ground yellow corn, 50 parts ground oats, 50 parts wheat bran, 25 parts soybean oil meal and 2.25 parts iodized salt. During the experiment they were fed and milked twice daily, accurate daily records were kept of feed consumption and milk production, and a 2-day composite milk sample saved each 10-day period for DDT and butterfat determinations. The amount of DDT in the milk was determined by the colorimetric method of Schecter *et al.* (13).

For the study comparing the concentration of DDT in milk when the DDT was administered by various methods, the animals were fed by one method for approximately 50 days, followed by other similar periods with different methods of administration at the same daily DDT intake. The DDT-in-oil solution was prepared as a 10 per cent solution in soybean oil. This oil solution was fed in two ways: (a) as a measured amount of oil in gelatin capsules given twice daily and (b) as a part of the grain ration for which the proper amount of oil solution was thoroughly mixed with the grain and fed twice daily. The crystalline DDT was similarly fed as (a) the desired dosage of crystalline material in gelatin capsules given twice daily and (b) crystalline DDT thoroughly mixed in a part of the grain ration and fed twice daily.

Concentrations of DDT in the milk of cows fed varying dosages of technical

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DDT as crystalline material were compared with the previously reported concentration of DDT in the milk of cows fed forage containing DDT residues (6, 8, 15).

The DDT in "soybean oil solution plus detergent" was a 10 per cent solution of DDT in soybean oil containing the same proportions[2] of two detergents[3] to DDT as were used during 2 yr. of forage field-spraying experiments.

RESULTS AND DISCUSSION

Since previous trials (8) have shown that higher concentrations of DDT were excreted in the milk of cows fed DDT-sprayed forage than when comparable amounts of an oil solution of crystalline DDT were fed, four different methods of administering technical DDT were studied to determine if significant differences might be observed in DDT excretion in the milk.

TABLE 1

The concentration of DDT in the milk of cows fed various dosage levels of DDT by four methods of administration

Cow no.	Wt.	Feeding period	Method of feeding	DDT intake		DDT in milk		% of DDT intake in milk
	(*lb.*)	(*d.*)		(*mg. daily*)	(*ppm. feed*)	(*ppm.*)	(*mg. daily*)	
N327	819	50	Oil (cap)[a]	50	4.5	0.13	0.9	1.8
N327	811	50	Oil (cap)	100	10.0	0.34	2.5	2.5
N327	815	50	Oil (Grain)[b]	100	10.0	0.42	2.2	2.2
N327	815	200	Oil (Grain)	100	10.2	0.46	4.1	4.1
N327	833	50	Cryst. (Grain)[c]	100	10.3	0.51	4.9	4.9
N277	1131	50	Cryst. (Cap)[d]	250	16.4	0.89	8.6	3.4
N618	683	50	Oil (cap)	250	26.6	1.1	7.2	2.9
N618	709	50	Oil (cap)	500	52.0	2.1	14.9	3.0
N277	1169	60	Cryst. (cap)	500	34.0	1.7	14.3	2.9
N277	1138	190	Cryst. (cap)	500	35.4	2.8	35.1	7.0
N277	1072	50	Cryst. (Grain)	500	33.8	2.6	29.1	5.8
N618	734	60	Oil (Grain)	500	53.0	2.4	15.3	3.1
N618	727	190	Oil (Grain)	500	54.2	3.3	29.8	6.0
N618	722	50	Cryst. (Grain)	500	68.3	2.0	19.9	4.0
N493	820	50	Oil (cap)	1,000	86.5	3.7	46.3	4.6
N143	1049	190	Oil (cap)	1,000	108.4	5.7	25.3	2.5
N493	841	60	Oil (cap)	2,000	146.0	6.0	74.5	3.7
N493	854	50	Oil (Grain)	2,000	154.0	6.7	75.0	3.8
N493	853	50	Cryst. (cap)	2,000	166.5	8.1	76.9	3.8
N493	865	140	Cryst. (cap)	2,000	184.0	8.5	66.3	3.3
N493	905	50	Oil (cap)	2,000	189.9	8.1	49.5	2.5
N493	925	50	Cryst. (Grain)	2,000	193.4	6.7	29.3	1.5

[a] A 10% solution of technical DDT in soybean oil administered in a gelatin capsule.
[b] A 10% solution of technical DDT in soybean oil thoroughly mixed with grain.
[c] Crystalline technical DDT thoroughly mixed with grain.
[d] Crystalline technical DDT administered in gelatin capsules.

The concentration of DDT in 4 per cent fat-corrected milk (F.C.M.) when fed at different levels of intake and by four methods of administration is presented in table 1. Most of the feeding periods were 50 or 60 days in length;

[2] 25 ml. Triton X-155, 25 ml. Triton X-1956, 1 lb. technical DDT.

[3] Triton X-155 (alkyl phenoxy polyethoxy ethanol) and Triton X-1956 (a phthalic glycerol alkyl resin).

however, several periods were longer. Differences in the DDT concentrations in the milk when fed by the different methods studied are small compared to the range of values secured with the same method of administration at a given dosage level, and are certainly not of the magnitude reported (8) for differences between feeding as a residue on sprayed forage or as an oil solution of technical DDT. It is concluded that there were no consistent differences in the excretion of DDT in the milk when the DDT was fed by these four methods of administration.

Examination of the data for certain animals receiving a constant dosage level for longer periods might indicate that continued dosage would increase the concentration of DDT in 4 per cent F.C.M.; however, the increased concentrations of DDT in the 4 per cent F.C.M. observed when feeding cows N277 and N618 500 mg. of DDT daily for 190 days as compared to the 60-day feeding period are primarily due to higher concentrations of DDT being excreted in the milk after parturition when DDT feeding was continued during the dry period. The 60-day feeding period for these animals was prior to the dry period. Cow N493, fed 2000 mg. daily of crystalline DDT in capsules for 50 and 140 days, did not show this increase in concentration of DDT in 4 per cent F.C.M. with continued feeding, because the longer period did not include a period following parturition.

Unpublished data from this laboratory indicate that the concentrations of DDT in 4 per cent F.C.M. remains fairly constant throughout lactation, except for increases following parturition when DDT was fed during the dry period, while the total daily excretion of DDT varies directly with the daily butterfat production. The butterfat probably represents a rather small percentage of the total fat pool of the body to which DDT is distributed.

A comparison of DDT excretion in the milk of cows when the DDT was fed at various dosage levels as (a) a residue on field-sprayed forage in previous studies (6, 8, 15) or (b) as crystalline DDT in the present study, is presented in figure 1. A regression line was calculated for each set of data used. It is possible to calculate the predicted concentration of DDT in 4 per cent F.C.M. when feeding DDT sprayed forage from the formula for the regression line ($Y = 0.486 + 4.149X$). Similarly, the concentration of DDT in 4 per cent F.C.M. when feeding a certain dosage level of crystalline DDT may be calculated from the formula for this regression ($Y = 0.59 + 1.27X$). Higher concentrations of DDT were excreted in the milk of cows fed DDT as a residue on sprayed forage than when comparable dosages were fed as crystalline DDT. For example, the estimated concentrations of DDT in the milk when 2 mg. of DDT per kilogram of body-weight are fed by each of these methods indicates that when the DDT is fed as a forage residue the concentration in the milk is approximately three times as great as when crystalline DDT is fed. These differences suggest that possibly (a) intestinal absorption of DDT residues from sprayed forages is more complete than by other methods of administration or (b) more DDT may be stored in the body fat when fed as the pure compound while a smaller proportion is excreted in the milk fat.

Work reported by Harris *et al.* (10) has shown that sheep fed DDT-dusted alfalfa stored greater amounts of DDT in mesenteric and kidney fat than when

fed equivalent amounts of crystalline DDT in capsules. Since differences in excretion of DDT in the milk fat were found similar to those reported by Harris *et al.* (10) for the storage of DDT in the body fat of sheep, the indication is that body fat storage of DDT in the dairy cow might parallel excretion of DDT in milk fat when fed by these two methods.

Since the material for the sprayed alfalfa used in this study was prepared with the aid of two detergents, an investigation of the effect of these detergents on the concentration of DDT in the milk was made. Five cows receiving four dosage levels of DDT were fed 10 per cent DDT in soybean oil solution for 50 to 60 days and then a 10 per cent soybean oil solution of DDT containing the same

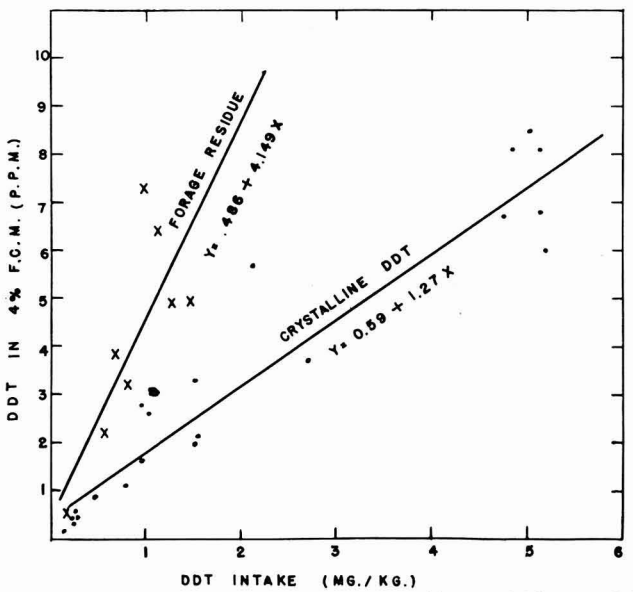

FIG. 1. Excretion of DDT in milk when fed as a residue on field-sprayed forage and as crystalline DDT.

proportions of the two detergents to the DDT as were used in the field-spraying experiments. Both solutions were administered in gelatin capsules. The concentrations of DDT in the 4 per cent F.C.M. for each cow at the various levels of DDT intake are presented in table 2. Concentration of DDT in the milk is not appreciably different when the DDT in "soybean oil plus detergent" was fed than when DDT in soybean oil alone was fed at the same dosage level. Frawley and Fitzhugh (9) have reported large differences in the fat storage of the beta isomer of benzene hexachloride in rats when 3 per cent of the dietary fat was replaced with an emulsifier (Tween 20). It is well known that other emulsifying agents affect the intestinal absorption of certain fat-soluble substances; however, the concentration of detergents employed in our study was much lower than that used by Frawley and Fitzhugh (9). The percentage of fat in the diet also has

been shown to affect the absorption and storage of DDT (12) and benzene hexachloride (9). The maximum amount of additional fat fed to the dairy animals in this study was 20 ml. daily of soybean oil which is a small addition to the fat content of a normal dairy ration.

SUMMARY

Higher concentrations of DDT in the milk occurred when cows were fed DDT as a residue on field-sprayed alfalfa than when comparable dosages of crystalline DDT were fed, suggesting that possibly intestinal absorption of DDT residues from sprayed forages is more complete than by other methods of administration, or that more DDT might be stored in the body fat when fed as the crystalline material while a smaller proportion is excreted in the milk fat.

There were no consistent differences in the excretion of DDT in the milk when the DDT was fed as a soybean oil solution either in capsules or mixed with grain, or as crystalline DDT fed either in capsules or mixed in grain.

TABLE 2

The concentrations of DDT in the milk of cows fed DDT in oil solution and DDT in oil solution plus detergent

Animal	Daily dosage	DDT in 4% F. C. M. (ppm.)			
		Oil solution only		Oil solution + detergent	
		Av.	Range	Av.	Range
N327	100	0.56	(0.41– 0.73)	0.52	(0.30– 0.80)
N277	500	3.66	(3.37– 4.23)	3.33	(2.62– 3.76)
N618	500	3.02	(2.09– 3.79)	3.57	(3.41– 3.70)
N143	1,000	8.24	(5.71–10.90)	8.39	(6.36–10.47)
N493	2,000	9.21	(8.07–10.92)	7.89	(6.09–10.15)

Additions of small amounts of detergents to the soybean oil solution of DDT had no effect on the concentration of DDT in the milk.

Increasing dosage levels of crystalline DDT gave progressive increases in the DDT concentration of the milk in a straight line relation. Increasing intakes of DDT as a residue on sprayed alfalfa also gave a straight-line increase in DDT excretion in the milk but at a greater slope than with crystalline DDT. Estimated concentrations of DDT in the milk may be calculated for any intake and either method of administration from the corresponding slope of the regression line.

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SEPARATION OF α-, β- and γ-Casein[1]

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Casein was long considered to be a pure protein. However, it became apparent from the studies of Linderstrøm-Lang (6) and others that modification of this view was necessary. The electrophoretic investigation of Mellander (9) demonstrated that casein is composed of at least three components, which he designated α-, β- and γ-casein in the order of their decreasing mobilities. It was apparent from previous attempts to fractionate casein, as well as the constancy of its composition, that the separation of the electrophoretic components would be difficult. Warner (12), who devised the first chemical method for separating α- and β-casein, has reviewed previous methods for fractionating casein. His method depends on differences in the solubility of α- and β-casein in water at pH 4.4 and 2° C. This method, which requires repeated precipitations from dilute solutions, becomes tedious when large amounts of the purified casein components are desired. A method for the separation of γ-casein, based on its solubility in 50 per cent alcohol, has been reported recently by Hipp *et al.* (5).

This paper describes two methods suitable for separating the three components of casein in quantity. The first method is based on differences in their solubility in 50 per cent alcohol in the presence of salt, as well as in water, with changes in temperature and pH. The second method is based on their solubility in aqueous urea solutions at the isoelectric point of casein. The problem of devising a method for separating the three caseins involves finding conditions where their interactions are reduced.

EXPERIMENTAL

Acid casein was prepared from unpasteurized skimmilk by acidification, as described by Hipp *et al.* (5). When not used immediately, the wet casein was stored with toluol at about – 20° C. The progress of fractionation was followed electrophoretically, according to the Tiselius method. The method of calculating areas and mobilities was the same as that described by Warner (12). The electrophoretic pattern, shown in figure 1, a, indicates that unfractionated casein contains about 75 per cent α-casein, 22 per cent β-casein and 3 per cent γ-casein.

Measurements of pH in 50 per cent alcohol were made in the usual manner with the glass electrode. Although no absolute significance is attached to the pH values in 50 per cent alcohol, procedures based on these measurements are reproducible. The point of minimum solubility of unfractionated casein is about

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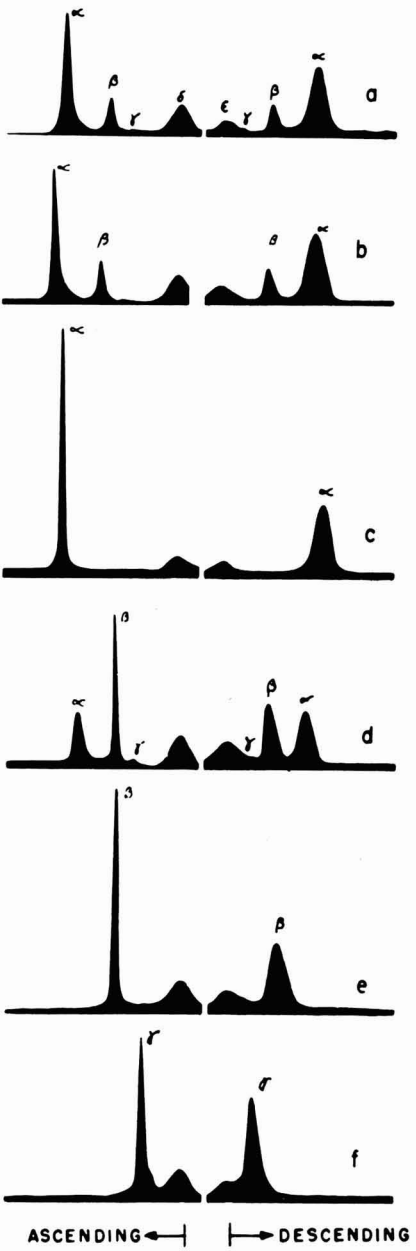

FIG. 1. Electrophoretic patterns obtained in a veronal buffer at pH 8.4 with an ionic strength of 0.1, containing 0.05 *N* NaCl, protein concentration 1%, at field strength of 4.33–4.86 volts/cm. after electrophoresis for 3 hr. (a) Unfractionated casein; (b) fraction B; (c) α-casein; (d) fraction C; (e) β-casein; (f) γ-casein.

pH 5.7 in 50 per cent alcohol, as compared with pH 4.7 in water. Fractionations were carried out at room temperature, (20–28° C.) unless otherwise stated.

Fractionation of casein in 50 per cent alcohol

Exploratory experiments showed that the solubility of isoelectric casein in ethyl alcohol-water solutions was greatest when the alcohol concentration was about 50 per cent. It was demonstrated also that the presence of salt is important in separating the electrophoretic components of casein with alcohol. As a preliminary step, casein was separated into three fractions, designated A, B and C, by adjusting the pH and the temperature of an alkaline solution of casein in 50 per cent alcohol containing 0.2 *M* ammonium acetate. Approximately 1,000 g. (dry weight) of wet casein were suspended in water and dissolved by the slow addition of 0.1 *N* NH_4OH to give a 6.6 per cent solution of casein of pH 7 when adjusted to a volume of 15 l. by adding water. Ammonium acetate (462 g.) then was added, making the solution 0.4 *M*, and an equal volume of absolute alcohol was added slowly, with stirring.

Fraction A. The alcoholic casein solution was adjusted to pH 6.5 by the slow addition of 1 *N* acetic acid in 50 per cent alcohol, precipitating fraction A. After standing for several hours or overnight, the precipitate was removed by filtration through 50-cm. fluted filter papers. The precipitate, amounting to 30 per cent of the total casein, contained 92 per cent α-casein and 8 per cent β-casein. It also contained the proteolytic enzyme of casein (13).

Fraction B. The filtrate obtained from fraction A was adjusted to pH 5.7 by adding 2 *N* acetic acid in 50 per cent alcohol. Stirring was continued for 1 hr. in order to collect the gummy precipitate. After standing for 3 hr. or overnight, the insoluble precipitate, fraction B, was filtered on large fluted papers. This fraction, amounting to 39 per cent of the total casein, contained 80 per cent α-casein and 20 per cent β-casein.

Fraction C. The protein in the filtrate from fraction B was precipitated by cooling to 2° C. An alternate, though less desirable method, is to dilute with an equal volume of water at room temperature. This fraction, amounting to 31 per cent of the total casein, contained 44 per cent α-casein, 50 per cent β-casein and 6 per cent γ-casein.

Preparation of α-casein. Electrophoretically pure α-casein may be prepared from either fraction A or B. It was prepared more easily from fraction A, but, when prepared from this fraction, it contained the proteolytic enzyme and when dissolved gave a slightly turbid solution.

In preparing α-casein from fraction A, the wet precipitate was suspended in water and dissolved by adding 0.1 *N* NH_4OH to give a solution of pH 7.2. The protein concentration was adjusted to about 6 per cent by diluting with water. A volume of absolute alcohol equal to that of the water used then was added, increasing the apparent pH of the solution to 7.7. Electrophoretically, pure α-casein was precipitated by the slow addition of a solution of 2 *M* ammonium acetate in 50 per cent alcohol until the pH was 7.2; at this point a definite granular precipitate formed. Usually, about 25–30 ml. of ammonium acetate

solution were required for each liter of solution. The precipitate was removed by centrifugation and washed at least three times with 50 per cent alcohol containing 0.07 *M* ammonium acetate. To make α-casein isoelectric, the precipitate was dissolved with dilute NH_4OH and reprecipitated with dilute acetic acid at pH 4.7 and washed free of salt. The yield of dry α-casein was about 15 per cent, based on the weight of the unfractionated casein used.

This procedure did not give pure α-casein when applied to fraction B. Pure α-casein could be prepared, however, by dissolving fraction B in borax and precipitating it in 50 per cent alcohol at pH 5.7 in the presence of 0.15 *M* NaCl. Two kg. of wet fraction B precipitate (800 g. dry weight) were suspended in water and dissolved by the addition of 280 g. of borax and adjusted to 13 l. with water, giving a 6 per cent protein solution at pH 8. NaCl (228 g.) and 13 l. of absolute alcohol were added, making the solution 0.15 *M* NaCl and approximately 50 per cent alcohol. The protein then was precipitated at pH 5.7 by adding 2.1 l. of 0.5 *N* HCl in 50 per cent alcohol. After standing overnight, the supernatant was removed by decantation. The sticky precipitate was washed with 2 l. of 50 per cent alcohol containing 0.15 *M* NaCl. This fractionation was repeated twice, with the exception that the volumes used were reduced to one-half the preceding volume. A yield of 285 g. of α-casein was obtained from 800 g. of fraction B, or about 15 per cent, based on the original casein used.

Preparation of β-casein. β-casein is concentrated in fraction C. This fraction contained about equal parts of α- and β-casein and all the γ-casein. Pure β-casein was separated from the other components by utilizing the difference in solubility of the three components in water at 2 and 25° C. (12). Fraction C (1 kg.) was suspended in water and dissolved with a minimum quantity of dilute NaOH and diluted to a protein concentration of about 1 per cent. After it was cooled to 2° C. cold dilute HCl was added dropwise to the solution, with constant stirring, until the pH was 4.5. The precipitate formed under these conditions was largely α-casein, which was removed by filtration at 2° C. Crude β-casein was precipitated from the filtrate by warming to 32° C. and it then was removed by filtration, leaving most of the γ-casein in the filtrate. This filtrate, as well as the second filtrate obtained in a similar manner, was used for preparing γ-casein.

After two extractions at 2° C. and pH 4.5, the insoluble fraction was free of γ-casein and was used as a source of β-casein. It was dissolved to make a 1 per cent solution and then was reprecipitated five times at pH 4.3 and 2° C. A total yield of 115 g., containing 98 per cent β-casein, was obtained by warming these filtrates to 32° C. Three further extractions of the insoluble residue at pH 4.0 yielded 160 g. of 90 to 95 per cent β-casein. Pure β-casein was obtained by reworking the fractions containing more than 90 per cent of this component. The α-casein impurity was removed by precipitation from a 0.1 per cent solution at pH 4.4 and 2° C. The filtrate was adjusted to pH 4.9, and β-casein was precipitated by warming to 32° C. After dissolving and filtering, the product was precipitated at its isoelectric point of pH 4.9 and washed free of salt. It is advantageous to use acetone followed by ether in drying the product, rather than

alcohol, since β-casein is soluble in alcohol-water solutions. A total of 166 g. of electrophoretically pure β-casein (figure 1, e) was obtained.

Preparation of γ-casein. The first two filtrates obtained in preparing crude β-casein by precipitating from solution at 32° C. were used for preparing γ-casein. Electrophoretic patterns indicated that this fraction contained 44 per cent γ-casein, 12 per cent β-casein, and 44 per cent of a component of casein moving more slowly than γ-casein. The total protein was precipitated from these filtrates by adding enough NaCl to adjust to 0.5 *M* and removed by filtration. The precipitate was dissolved from the filter paper in dilute NaOH and reprecipitated from a volume of 1500 ml. in the presence of 0.5 *M* NaCl by the addition of acid until the pH was 5.0. The precipitate, containing about 30 g. protein, was collected by means of centrifugation. It then was dissolved in dilute NaOH and dialyzed in the cold to free it of NaCl. The crude γ-casein solution, with a volume of 200 ml., was adjusted to pH 6.4 by addition of 0.01 *N* HCl at room temperature. The protein was largely precipitated as a sirup. Thirteen g. (dry basis) of the sirupy precipitate were extracted three times with 500 ml. of 50 per cent alcohol at 35° C. and cooled to room temperature. The insoluble material was removed by centrifugation. After addition of 500 ml. of 50 per cent alcohol to clarify the combined supernatant, the product was precipitated by cooling to 2° C. After standing for a day, the precipitate was removed by centrifugation at 2° C. γ-Casein then was dissolved in 2 l. of 50 per cent alcohol at room temperature and reprecipitated at 2° C. as before. The precipitate was removed by centrifugation at 2° C., and washed with 20 volumes of acetone. The product then was dried with a mixture of equal volumes of acetone and ether at room temperature. A yield of 6 g. of pure γ-casein having the electrophoretic pattern shown in figure 1, f was obtained.

Fractionation of casein by means of aqueous urea solutions

Whole casein, such as that freshly prepared from skimmilk or the dried product, when dissolved in concentrated aqueous urea solutions, can be separated into its components by merely adding water in suitable amounts (figure 2, a, b and c). Thus, when the concentration of urea was reduced to 4.6 *M* by the addition of water, α-casein became insoluble. After the removal of residual α-casein by the addition of water, β-casein was precipitated from the filtrate by making the urea concentration 1.7 *M*. γ-Casein remained in solution and was removed by further dilution with water or by the addition of $(NH_4)_2SO_4$. The process is operable at ordinary room temperature.

The following example illustrates the method of separating the components of casein by means of urea solutions:

Fifteen hundred g. of wet casein (400 g., dry weight) were dissolved by addition of 1,500 g. of urea and water, making a total volume of 3.75 l. and giving a urea concentration of 6.6 *M*.

α-Casein. The solution was warmed to room temperature, and 1.65 l. of water were added slowly, with stirring, to the casein-urea solution, making the concentration of urea 4.63 *M*. The precipitate formed under these conditions was

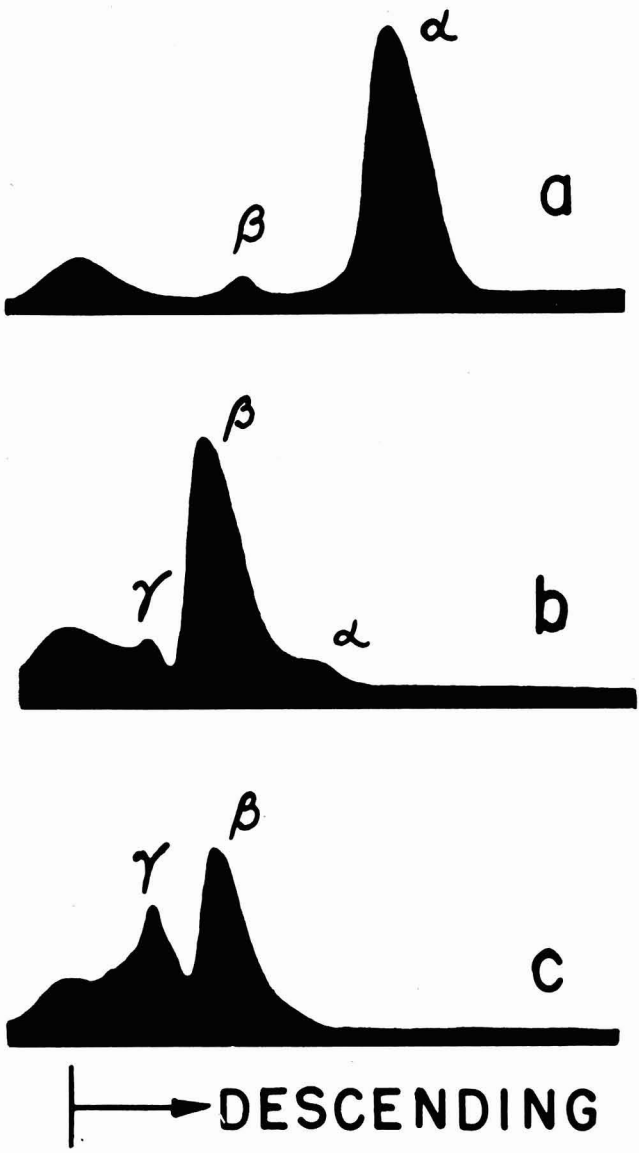

FIG. 2. Electrophoretic patterns obtained in a veronal buffer at pH 8.4 with an ionic strength of 0.1, containing 0.05 *N* NaCl, protein concentration 1%, at a field strength of 4.56–5.05 volts/cm., after electrophoresis for 3 hr.; (a) Insoluble in 4.6 molar urea; (b) soluble in 3.3 and insoluble in 1.7 molar urea; (c) soluble in 1.7 molar urea.

largely α-casein (figure 2, a), which was removed by centrifugation. The crude α-casein was further purified by dissolving it in 1,500 ml. of 6.6 *M* urea containing 15.9 g. of NaCl. α-Casein was precipitated by the addition of 1,500 ml. of water.

Further impurities were removed by washing the precipitate with a 4.7 *M* urea solution. In order to remove a trace of β-casein, the product again was dissolved in urea and reprecipitated as before. Urea was removed from the product by washing with an excess of water. After drying, 180 g. of pure α-casein were obtained, a calculated yield of 60 per cent, based on the α-casein content of the whole casein used. This α-casein contained a small amount of proteolytic activity (13).

β-Casein. The filtrate, obtained after the removal of the precipitated α-casein at 4.63 *M* urea, was diluted to 3.3 *M* urea by the addition of water. The small insoluble precipitate obtained here was a mixture of α- and β-casein in about the proportion in which they occur in unfractionated casein; it was removed by centrifugation and discarded. The portion soluble in 3.3 *M* urea was diluted to 1.7 *M* urea, and the pH was adjusted to 4.7 by addition of about 20 ml. of 0.1 *N* HCl. Seventy-eight g. of crude β-casein, (figure 2, b) were precipitated. The crude β-casein was purified by dissolving it in 2 l. of 4.6 *M* urea and fractionating by dilution with water. Again, the portion soluble is 3.3 *M* urea but insoluble in 1.7 *M* urea contained the purest β-casein. By reprecipitation in this manner, the remaining small amount of α-casein was removed. Thirty-two g. of pure β-casein were obtained, a yield of 40 per cent, based on the total β-casein in the whole casein used. By reworking the material insoluble in 3.3 *M* urea obtained in purifying β-casein, the yield of β-casein can be increased.

γ-*Casein.* The casein soluble in 1.7 *M* urea, obtained from the supernatant of the crude β-casein precipitate, was precipitated by adding solid $(NH_4)_2SO_4$ to the solution until the concentration of $(NH_4)_2SO_4$ was 1.6 *M*. The casein was precipitated completely and separated by filtration. From 20 to 30 g. of the dried salt-free casein were obtained. This fraction contained 40 per cent γ-casein contaminated with some impurity and 60 per cent β-casein (figure 2, c). The γ-casein was further concentrated by dissolving this material in 250 ml. of 2 *M* urea and removing impurities by warming the solution to 60° C. and diluting to 1 *M* urea. The precipitate formed, which was removed by centrifugation, contained large amounts of β-casein. The γ-casein fraction was precipitated from the supernatant by adding solid $(NH_4)_2SO_4$. By repeating this procedure of dissolving and reprecipitating the portion insoluble in 1.0 *M* urea, the yield of γ-casein can be increased. The material precipitated with $(NH_4)_2SO_4$ contained 80 per cent γ-casein and 20 per cent β-casein. Pure γ-casein was obtained by dissolving this product in dilute NaOH and precipitating the impurities at pH 4.7 and 2° C. The supernatant was adjusted to pH 5.8 at 2° C., and γ-casein was precipitated by warming to 30° C. About 3 to 4 g. of pure γ-casein were obtained, a yield of 25 per cent, based on the γ-casein in the starting material.

DISCUSSION

Several methods have been described previously for fractionating casein by means of alcohol. The extensive method of Linderstrøm-Lang (7) is based on separations in acid-alcohol solutions at relatively high temperatures. Although no evidence was given for the homogeneity of fractions prepared by the acid-

alcohol method, the variations in phosphorus content of the fractions indicated separation. It appears likely that Linderstrøm-Lang's fraction K_1, containing 0.1 per cent phosphorus and amounting to 3 per cent of the original casein, contained a large amount of γ-casein. No attempt was made to prepare γ-casein by Linderstrøm-Lang's procedure. It was found (12), however, that the separation of α- and β-casein by his method was slight, as indicated by electrophoresis.

Groh *et al.* (4) fractionated casein from phenol, urea-alcohol and 70 per cent alcohol solutions. Their fractions were characterized by the tyrosine, tryptophane and phosphorus contents, as well as by optical rotation. The values for the tyrosine and tryptophane contents of their K_1 fractions are in good agreement with Gordon *et al.* (2) for α-casein. Their values for the specific rotation of their K_1 and K_2 fractions also are in good agreement with the values reported by Hipp *et al.* (5) for α- and β-casein, respectively. However, their values for the phosphorus content of fractions K_1 and K_2 indicate incomplete separation when compared with the phosphorus content of α- and β-casein. Our experience with the ammoniacal 70 per cent alcohol method of Groh *et al.* (4) indicates that by their method pure α-casein can be prepared from their K_1 fraction in small yields, but that their K_2 fraction contained only about 80 per cent β-casein.

The present method of separating the components of casein by alcohol depends in part on variations in the solubility of the components in 50 per cent alcohol. The efficiency of separation, however, was greatly increased by the presence of salt. Both these solubility effects are consistent with the amino acid composition of α- and β-casein, as determined by Gordon *et al.* (2). They found that α-casein contained 548 polar groups and 341 nonpolar groups, whereas β-casein contained 465 polar groups and 442 nonpolar groups per 10^5 g., showing that β-casein is more nonpolar than α-casein; consequently, both alcohol and salt are effective in their separation. The amino acid analyses of γ-casein are incomplete as yet, but the proline content was found to be 17 g. per 100 g. protein, as compared with 15.1 g. in β-casein and 7.47 g. in α-casein (3).

The high proline and low phosphorus contents of γ-casein are consistent with its high solubility in alcohol. The solubility of α-, β- and γ-casein in water and 50 per cent alcohol has been reported previously (5). γ-Casein and β-casein are much more soluble in water and in 50 per cent alcohol than is α-casein. Moreover, β- and γ-casein are at least fifteen times more soluble in water at 2.5 than at 25° C. These similarities in properties of β- and γ-casein indicate similarities in structure.

Separation of the electrophoretic components of casein by means of aqueous urea was particularly effective. An inexpensive purified grade of urea was used. The separations can be made from concentrated casein solutions, making preparation of the components in quantity convenient. Burk and Greenburg (1) found that molecular weight of unfractionated casein in 6.6 *M* urea is 33,600. Since this value is much lower than the value of 75,000 to 100,000 reported by Svedberg *et al.* (11), it has been thought that urea splits the casein molecule (10). The pure components of casein separated by means of urea have the same composition and properties (table 1) as when separated by changes in pH or alcohol,

indicating that urea does not split the casein molecule. Groh *et al.* (4) devised a method of separating casein by means of urea and alcohol. The use of aqueous urea solutions by the method herein described is less complicated and results in the separation of electrophoretically pure components.

It was noted by Warner (12) that the electrophoretic pattern of the rising boundary of α-casein frequently showed two peaks at pH values alkaline to the isoelectric point. In the present work, the rising boundary of α-casein showed only one peak. This difference can be accounted for by the fact that the casein used by Warner was obtained from the milk of one or two cows, whereas the casein herein described was from mixed milk obtained from a large number of animals. McMeekin *et al.* (8) have shown that the electrophoretic pattern of the α-casein component from individual cows occasionally has two peaks. The reason

TABLE 1

Composition and properties of the components of casein prepared by various methods

Method of preparation	N[a, b]	P[a]	S[a]	Mobility[c]	Specific[d] rotation	Intrinsic[e] viscosity
	(%)	(%)	(%)	(u)	$([\alpha]_D^{25})$	([η])
Isoelectric, 2° C.						
α	15.56	0.982		−6.64	− 88.2	0.105
β	15.39	0.602				
Aqueous alcohol						
α	15.58	0.99	0.72	−6.75	− 90.5	0.100
β	15.33	0.55	0.86	−3.05	−125.2	0.147
γ	15.40	0.11	1.03	−2.01	−131.9	0.095
Aqueous urea						
α	15.57	0.98		−6.50	− 87.4	0.105
β	15.47	0.64		−3.15	−124.6	0.145
γ	15.59			−2.04		

[a] On a moisture-free basis.

[b] We are indebted to Mary Jane Welsh for making the nitrogen determinations.

[c] In veronal buffer at pH 8.4, $\mu = 0.1$, containing 0.05 *N* NaCl, 1% solution, calculated from descending boundary in $cm^2\ volt^{-1}\ sec^{-1} \times 10^5$.

[d] Determined on 1% solution in pH 8.4 veronal buffer, $\mu = 0.1$. A value of −105 was obtained for unfractionated casein.

[e] Determined in 0.05 *N* NaCl at pH 6.4 to 6.9, (concentration in g./100 ml. of solution). These values for intrinsic viscosity are an indication of molecular size.

for this difference in the casein from individual cows is obscure. No difference was noted in the composition of α-casein with one electrophoretic peak and that of α-casein with two peaks.

SUMMARY

Two methods are described for separation of the electrophoretic components of casein. In the first method, the separation is accomplished by precipitation from 50 per cent alcohol solutions of casein by means of variations in temperature, pH and ionic strength and by isoelectric precipitation from water.

The second method is based on the solubility of the casein components in aqueous urea. The urea method is relatively simple and gives products with the same composition and properties as those obtained by the pH and also the 50 per cent alcohol methods.

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ABSTRACTS OF LITERATURE

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BOOK REVIEWS

89. Advances in Enzymology, Vol. 11. F. F. NORD, editor. Interscience Publishers, Inc., New York, N. Y. 471 pp. 1951.

The chapters are as follows: The nature of entropy and its role in biochemical processes, by H. Gutfreund; Reactions at interfaces in relation to biological problems, by J. F. Danielli and J. T. Davies; Chlorophyll fluorescence and photosynthesis, by E. C. Wassink; Thiol groups of biological importance, by E. S. Guzman Barron; Pectic enzymes, by H. Lineweaver and E. F. Jansen; Enzymic synthesis of polysaccharides: A biological type of polymerization, by E. J. Hehre; The biological transformations of starch, by S. Peat; Chemical investigations on alliin, the specific principle of garlic, by A. Stoll and E. Seebeck; and Some problems of pathological wilting in plants, by E. Gäumann. The character is definitely international, since 6 of the chapters are by European scientists.

Author and subject indices of this volume, as well as a table of contents of the 11 volumes of the series which have appeared so far, seem to be quite adequate. F. E. Nelson

90. Advances in enzymology, Vol. 12. F. F. NORD, editor. Interscience Publishers, Inc., New York, N. Y. 570 pp. 1951.

Contrary to the usual custom, this volume is the second of the series issued in 1951. The chapters include Oxidoreduction in chloroplasts, by R. Hill; Mechanism of fixation of carbon dioxide by heterotrophs and autotrophs, by M. F. Utter and H. G. Wood; Enzyme-substrate compounds, by B. Chance; The specificity of certain peptidases, by E. Smith; The enzymic hydrolysis and synthesis of acetylcholine, by D. Nachmansohn and I. B. Wilson; The present status of starch chemistry, by K. H. Meyer and G. C. Gibbons; Enzymes of starch degradation and synthesis, by P. Bernfeld; Biological methylation, by F. Challenger; Reaction of borate with substances of biological interest, by C. A. Zittle.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

91. An investigation of pleuropneumonia-like organisms isolated from the bovine genital tract. D. G. ff. EDWARD, Welcome Vet. Research Sta., Frant, Sussex. J. Gen. Microbiol., **4,** 1: 4–15. 1950.

Two members of this group of organisms were isolated from the genital tract of cattle. The "S" strain appeared to be saprophytic and hence a contaminant of the discharges from the tract. The "P" strains appeared to be involved in an inflammation of the genital tract which seemed to be connected with infertility. These latter strains required the presence of blood serum to grow on agar. J. J. Jezeski

92. A biochemical study of experimental Q fever infection in the bovine mammary gland. R. A. ORMSBEE, Rocky Mtn. Lab., Pub. Health Service, Hamilton, Mont. Public Health Reports, **66,** 51: 1685–1693. Dec. 21, 1951.

Studies were made of the changes in composition of bovine milk following experimental infection, *via* the lacteal duct, with *Coxiella burnetii.* Four experimental animals were injected with infected chick yolk-sac tissue while 2 control animals received normal yolk-sac suspensions; 2 quarters of each animal were injected. Total nitrogen, non-casein nitrogen, chloride, butterfat, nonfat solids and pH values of the milk increased, while casein, lactose, milk volume and water decreased. These changes were found in milk from both groups of animals, but magnitude and duration of the changes were greater in that from the experimental animals. Even in this group, however, the major fluctuations in these constituents, as well as any fever, disappeared by the 8th day after injection. No fever was found in the control animals. The authors conclude that the experimentally induced Q fever infection caused these greater fluctuations in the constituents of milk from the experimental animals. D. D. Deane

93. A brucellosis survey of Brant County, Ontario. N. V. SANDERSON, R. H. KARN, W. R. LEGROW and O. C. RAYMOND. Can. J. Public Health, **42,** 7: 295–298. July, 1951.

The ring test was performed on a total of 1,039 composite milk samples of which 176 gave positive results. These herds were blood tested for brucellosis and, where reactors were found, persons who had been exposed were asked to submit to a blood test. Of 326 persons blood-

tested, 40 were positive, although only 2 of these had undulant fever symptoms. O. R. Irvine

94. Use of the ring test in the diagnosis of bovine brucellosis. H. L. GILMAN, N. Y. State Vet. Coll., Ithaca, N. Y. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 51–62. 1951.

This test is known as the ABR or Abortus Bang Ring Test discovered in 1937. The test is widely used in Scandinavian countries and in Finland. Since 1947, Demark has accredited herds as free from brucellosis on the basis of 3 consecutive negative ring tests of milk can samples followed by 1 negative blood test.

In the United States, results in Minnesota on on 8,469 herds show an over-all efficiency of 68% in locating infected herds by the ring test as compared with Danish reports of 82%.

The ring test has the merit of being simple and easy to make without the need for blood samples. Frozen milk, colostrum and possibly mastitis milk give false results. It may be affected by vaccination and cannot be applied to bulls, heifers and dry cows. The ring test is a valuable supplement to blood testing but it should not be used alone. Unless used as an adjunct to blood testing the ring test may lead to confusion. A. C. Dahlberg

BUTTER

O. F. HUNZIKER, SECTION EDITOR

95. Experiments on the packing and storage of butter. IX. The effect of pro-oxidants on the storage life of butter. C. R. BARNICOAT, Dairy Research Inst., Palmerston North, New Zealand. New Zealand J. Sci. Technol., **32A,** 2: 11–18. Aug., 1950.

The partition of Fe between butter and buttermilk was about as expected, but the concentration of Cu in the butter fraction was about 6 times that anticipated. Partitioning of Fe and Cu was unaffected by cream acidity. Traces of the 2 metals (<1 ppm.) caused deterioration of flavor during storage. Fe led to a metallic flavor that usually disappeared during storage. Cu catalyzed oxidation of the triglycerides, especially in the more acid butters, and sometimes caused tallowy flavors due to the oxidation of unsaturated fatty acids in the butterfat.
W. C. Frazier

96. Experiments on the packing and storage of butter. X. Experiments on predicting the storage life of butter. C. R. BARNICOAT, Dairy Research Inst., Palmerston North, New Zealand. New Zealand J. Sci. Technol., **32A,** 1: 37–43. June, 1951.

Predicting storage life or kind of deterioration of experimental or commercial butters was not feasible on the basis of their acidities, salt content or Cu and Fe content. Storage of butter at higher-than-normal temperatures did not enable predictions of storage life to be made. A promising, rapid "oven" sorting test is described for detection of incipient oxidation or presence of traces of Cu and Fe responsible for the defect. The butter is held in a glass dish, so that there is a defined ratio of surface area to depth of butter, in an oven at 110° C. for 4 hr.; then fat aldehyde values are determined by Schibsted's method and used as the sorting test.
W. C. Frazier

97. Influence des albumines sur la stabilitè du buerre (Influence of albumins on the stability of butter). P. DIATCHENKO. Lait, **31,** 308: 505–510. Sept.–Oct., 1951.

Deterioration in flavor and odor of various butter samples during storage was studied by organoleptic evaluation of fat and serum and increase in amino nitrogen of the serum. Putrefactive and cheesy types of off-flavor were detected in the serum rather than the fat and their presence was correlated with increases in amino nitrogen. S. Patton

98. Quality of vegetable parchment wrappers in relation to the quality of butter. W. R. MUMMERY, F. H. MCDOWALL and A. K. R. MCDOWELL, Dairy Research Inst., Palmerston North, New Zealand. New Zealand J. Sci. Technol., **32A,** 3: 1–14. Oct., 1950.

Investigations of the causes of off-flavor in butter due to a parchment paper indicated that a high content of Cu and Fe might be responsible for part of the defective flavor. Parchment papers containing more than 3 ppm. of soluble Cu or 6 ppm. of soluble Fe were likely to cause off-flavors in butter. W. C. Frazier

99. Methods of production of high quality whey butter. L. C. THOMSEN. Can. Dairy and Ice Cream J., **30,** 10: 36–38, 56. Oct., 1951.

With adequate precautions, whey butter can be made equivalent to sweet cream butter in all but keeping properties. The author discusses in detail the production of quality whey butter under the headings: (a) handling whey, including the skimming procedure; (b) handling whey cream; and (c) churning whey cream. H. Pyenson

100. Electric coolers for maintenance of cream quality on the farm. E. M. SHERWOOD and A. W. FARRALL, Mich. State College, East Lansing. Am. Dairy Prod. Mfg. Rev., **13,** 10: 12, 14, 53. Oct., 1951.

The electric cream cooler developed at Michigan State College is of the dry-box type. Tests showed that cream stored in the cooler developed about half the acidity of cream held under "normal farm methods". The flavor score was approximately 1 point higher. Cost of operation depended on the amount of cream cooled and ranged from 4.5 kwh-20.3 kwh/wk. It is suggested that cost of cooler and operation might be paid for from increased income from higher quality cream. T. J. Claydon

101. Butter—a spread for bread. W. B. COMBS, Univ. of Minn., St. Paul. Am. Dairy Prod. Mfg. Rev., **13,** 12: 10, 12. Dec., 1951.

Since the greatest use of butter is as a spread for bread, spreadability is of vital importance. If butter will not spread, the consumer may look for a substitute. During the winter season butter

may possess poor spreading qualities. Certain modifications of the manufacturing procedure have been developed to minimize the effect of season. These practices involve churning and washing temperatures and are reviewed briefly.
T. J. Claydon

CHEESE

A. C. DAHLBERG, SECTION EDITOR

102. Reducing extraneous matter in Canadian cheddar cheese. D. B. GOODWILLIE. Can. Dairy and Ice Cream J., **30,** 12: 27, 38. Dec., 1951.

A laboratory for examination of extraneous matter in cheese was set up in Montreal in April, 1951. Samples as received are weighed, processed, filtered and the filter discs classified 1, 2, 3 or 4 according to standards. Discs classified 3 and 4 are examined under the microscope and an attempt made to identify the extraneous matter present. From May to Oct. 42,958 samples, representing as many vats, were examined. There was a sharp improvement in the percentage of samples graded below no. 2 from May until Sept. Oct. was one of the poorest months since the testing was started. This reversal may be due to weather conditions and and season. In Aug., a typical month, 85.48% of the sediment 3 and 4 was classified as vegetable matter, 8.52% as coal dust, 0.81% as insect parts, 1.30% as grit and dust particles, 0.32% as broom bristles, 1.62% as cloth fibres, 0.49% as hair, 0.16% as metal particles and 1.3% as wood fibres.
H. Pyenson

103. New product? Then uniformity is a must. Food Eng. Staff. Food Eng., **23,** 11: 76, 77, 165, 166. Nov., 1951.

Since uniformity in quality is essential in successful marketing of a new food product, Borden's spray-dried E-Z Cheez is controlled carefully during manufacture. The process generally is similar to that for bakers cheese up to the stage of drying. Little detail is given of the latter operation. Laboratory supervision during processing, followed by baking tests on the finished product, help to insure a standard uniform product.
T. J. Claydon

104. Enzymes in the cheese industry. Parts I and II. M. G. FARNHAM. Can. Dairy and Ice Cream J., **30,** 10: 32–33, 54, Oct., 1951; **30,** 11: 59–64. Nov., 1951.

The author gives a review of the knowledge of enzymes in relation to making of cheese, pointing out the limited application of enzyme systems on a commercial scale. H. Pyenson

105. Process for producing heavy curd. G. J. STREZYNSKI (assignor to The DeLaval Separator Co.). U. S. Patent 2,574,508. 14 claims. Nov. 13, 1951. Official Gaz. U. S. Pat. Office, **652,** 2: 400. 1951.

A process is described for making cheese curd from milk containing less than 5% fat by centrifugal separation at 90° F. of the acid-precipitated curd and whey. R. Whitaker

106. Dehydrated cheese products and method of making. L. GOOTGELD. U. S. Patent 2,576,597. 5 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1100. 1951.

Cheese curd particles, containing some whey, are freeze-dried to less than 2% moisture.
R. Whitaker

107. Trends in the cheese industry. E. W. GAUMITZ, Natl. Cheese Inst. , Am. Dairy Prod. Mfg. Rev., **13,** 11: 18, 20, 34, 35. Nov., 1951.

The cheese industry in the U. S. now utilizes about 10% of the milk produced on farms. The amount used has steadily increased during the past 20 yr. and further increase is to be expected. Industry trends are discussed briefly under exports, imports, consumption, pasteurization, rindless cheese, mechanization, utilization of whey and developments in retail handling.
T. J. Claydon

108. New Roquefort-type flavors. S. PATTON, Penn State College, State College. Food Eng., **23,** 12: 93, 157. Dec., 1951.

Use of mold-ripened cheeses in certain commercial food preparations has its limitations. To overcome the disadvantages, use of cheese flavor extracts and synthetic Roquefort-type flavors was investigated. Flavor concentrates from natural cheese were obtained by oil extraction or steam distillation. A mixture of methyl amyl ketone and butyric acid gave a satisfactory synthetic blue cheese flavor. T. J. Claydon

109. Cheese dispenser. F. J. UPRIGHT. U. S. Patent 2,580,864. 5 claims. Jan. 1, 1952. Official Gaz. U. S. Pat. Office, **654,** 1: 196. 1952.

A slicer for cutting cheese into measured quantities is described. R. Whitaker

110. Creaming cottage cheese. J. C. MARQUARDT, N. Y. State Dept. of Agr. and Mkts., Albany. Am. Dairy Prod. Mfg. Rev., **13,** 12: 20. Dec., 1951.

Creamed cottage cheese must contain at least 4% fat. Creaming mixtures usually contain from 14-20% fat and more than 20% solids. Mixtures that have been held cold for 12 hr. are most desirable. The dryness of the curd governs the type of creaming mixture employed. One part 20% cream to 6 parts curd or 1 part 14% creaming mixture to 4 parts curd give suitable results. Several suggestions are offered for mixing procedures. T. J. Claydon

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

111. "O_2 below 0.2" system guards quality status quo. J. L. BECKER and R. BECKER, Maple Island, Inc., Stillwater, Minn. Food Eng., **23,** 11: 91, 92, 143, 148. Nov., 1951.

The packaging system of Maple Island, Inc., for its powdered dairy products includes: (a) storage of products for packaging in drums under vacuum; (b) specially designed contamination-proof feed-in method; (c) double vacuumizing for uniform O_2-free filling; (d) automatically

controlled inert gassing of containers; (e) a generator unit right in the plant to provide all inert gas; (f) an integrated lab technic for detecting "leaks" and checking on O_2. Details of the various steps in the packaging process are given. T. J. Claydon

112. Spray-drying apparatus. S. T. COULTER, R. E. MONTONNA, A. S. KITZES (assignors to Regents of the U. of Minn.). U. S. Patent 2,576,-264. 4 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1013. 1951.

A spray drier is described for milk and other fluids, consisting of a long tube, with the air directed through the tube from a frusto-conical-shaped chamber to insure uniform parallel air flow in the tube. The milk to be dried is introduced at the entrance to the long tube. The drying gas can be dehumidified and recycled if desired. R. Whitaker

113. Atomizing apparatus. D. D. PEEBLES (assignor to Golden State Co., Ltd.). U. S. Patent 2,574,705. 2 claims. Nov. 13, 1951. Official Gaz. U. S. Pat. Office, **652,** 2: 452. 1951.

A centrifugal atomizing spray wheel is described for drying milk, etc. R. Whitaker

114. Spray drying equipment and method. D. D. PEEBLES and R. E. MEADE (assignors to Western Condensing Co.). U. S. Patent 2,575,119. 3 claims. Nov. 13, 1951. Official Gaz. U. S. Pat. Office, **652,** 2: 562. 1951.

A design for a spray dryer of the centrifugal-wheel type, suitable for spray drying dairy products, is described. R. Whitaker.

115. Apparatus for rapid solution and/or suspension of powdered solids. D. D. PEEBLES and G. P. HENSLEY (assignors to Golden State Co., Ltd.). U. S. Patent 2,580,316. 6 claims. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1173. 1951.

A small hand device for reconstituting powdered milk consists of a cylinder and piston with discharge orifices designed to cause violent agitation. R. Whitaker

116. Milk can punch. S. M. CODIGA. U. S. Patent 2,578,462. 1 claim. Dec. 11, 1951. Official Gaz. U. S. Pat. Office, **653,** 2: 536. 1951.

A device easily punches a hole in evaporated milk cans. R. Whitaker

117. Milk can punch. C. E. ENGLE. U. S. Patent 2,576,505. 2 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1076. 1951.

A lid is described which holds 2 punches which when pressed down on an evaporated milk can form 2 openings in the can with edges bent inward, 1 for pouring and the other for an air vent. R. Whitaker

118. Enzymatic hydrolysis of cheddar cheese whey protein for use in foods. J. P. MALKAMES, JR., H. E. WALTER, A. M. SADLER and O. S. SAGER, B.D.I., Washington, D. C. Am. Dairy Prod. Mfg. Rev., **13,** 12: 24, 31. Dec., 1951.

Modification of the method previously reported for hydrolysis of Swiss cheese whey protein has given satisfactory results with cheddar cheese protein. The chief differences are in temperatures and lengths of time involved. Details of the processes are given for precipitation of whey protein and its subsequent enzymatic hydrolysis. T. J. Claydon

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

119. Contribution au controle bactériologique du lait (Contribution to the bacteriological control of milk). C. G. MACRIS and C. G. TZIVANOPOULOS. Lait, **31,** 308: 487–501. Sept.–Oct., 1951.

The nitroreductase test, employing sulfanilic acid and α-naphthylamine reagents to determine the conversion of added nitrates to nitrites, was investigated as a method of evaluating the bacteriological quality of milk. Procedures for preparing reagents, applying and interpreting the test are given in detail. Results indicated that raw milk recovered aseptically as possible exhibits no nitroreductase activity during a 10-hr. incubation at 37° C. Boiling or pasteurizing milk destroyed its nitroreductase activity. Milks produced with very little care showed a level of nitrites after 2-hr. incubation which was approximately proportional to their bacterial count. The course of the formation of nitrites enabled a deduction as to whether coliform organisms constituted the majority present. The nitroreductase test appeared well adapted for classification of milks and for use as a quality control test. S. Patton

120. Coliform organisms in milk and milk products. C. J. BABCOCK. P.M.A., U.S.D.A., Washington, D. C. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 153–163. 1951.

The author presents a very excellent thorough review of the subject and raises the question of the use of the coliform test in other dairy products, such as ice cream, dry milk, etc. It is concluded that "the coliform test is well adapted for use in milk and milk products' plants to ascertain that plant equipment is properly cleaned and handled." It is necessary to carefully interpret the results to assure correct application. A. C. Dahlberg

121. De proef van Ringeling (The Ringeling Test). J. SMIT and A. ZOET. Netherlands Milk and Dairy J., **5:** 186–193. 1951.

For detection of *E. coli* in pasteurized milk the Ringeling test is widely used in the Netherlands. Five ml. of pasteurized milk are added to 50 ml. "acid broth", prepared from an extract of 500 g. of fat-free meat in 1000 ml. of water, to which 1% peptone and 0.5% NaCl are added. After incubating at 37° C. for 24 hr., a loopful is streaked on Endo plates. *E. coli* must be absent. The results of this test often are doubtful, since no standardization of the "acid broth" to a certain pH is prescribed. A weak acid reaction (pH 5.95) usually results. Acidification to various levels with H_2SO_4 was tried. A pH of 5.0 proved to

have a distinct advantage over lower and higher acidities. W. C. van der Zant

122. Improving a city's milk supply. M. M. COHN, Dept of Health, Schenectady, N. Y. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 29–36. 1951.

Although there is no single test to solve the sanitary milk problem which is so varied in its aspects, the resazurin test is a very useful tool. From 1947–1949 in Schenectady compliance of raw milk with bacterial standards has increased from 28.0–84.8%, pasteurized milk from 40.5–82.2% compliance with standard plate count and coliform count improved from 79.8–94.4% compliance.

The rennet resazurin test has been used on all individual cans of producers' milk and on mixed-can samples to indicate mastitis and high bacterial counts. The program is to test the milk and promptly do field work with producers. On the 2nd day cans of poor milk are rejected. The dairy industry is required to inspect farms annually, make monthly tests of producers' milk for sediment and bacteria, file veterinary certificates on health of herds and inspect farms delivering faulty milk. A. C. Dahlberg

123. Preservation of milk by the use of water-soluble chlorites. J. D. MACMAHON (assignor to Mathieson Chemical Corp.). U. S. Patent 2,575,670. 6 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 780. 1951.

Milk is preserved by a small amount of a non-toxic soluble chlorite. R. Whitaker

124. The effect of antibiotics on some characteristics of milk. F. W. GILCREAS and ISABELLE STEWART, N. Y. State Dept. of Health, Albany. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 37–49. 1951.

This study was prompted by the general use of antibiotics and sulfa drugs in controlling udder infections and the problems which they have caused by slow acid development in cheese making.

Samples of milk to which 5–50 units of penicillin/ml. were added were held at 5–10° C. for periods up to 24 hr. The bacterial counts were slightly reduced but not sufficiently to affect the grade of milk. The effect on coliform bacteria was erratic. The cup assay method of testing for penicillin in milk was used. In one case a herd treated with penicillin produced milk that was negative the day following treatment, in another herd the test showed 0.42 units/ml. in the mixed herd milk. Somewhat similar results were obtained with sulfadiazine and sulfapyridine.

These products did interfere with normal souring of milk and it is reasonable to conclude they should not be present in milk for human consumption. A. C. Dahlberg

125. The ability of single phage particles to form plaques and to multiply in liquid cultures. A. KLECZKOWSKI and J. KLECZKOWSKI, Rothamsted Exptl. Sta., Harpenden, Hertfordshire. J. Gen. Microbiol., **5,** 2: 346–356. 1951.

A bacteriophage and a susceptible strain of *Rhizobium trifolii* (clover nodule bacteria) were used in experiments to test the assumption that single phage particles initiate multiplication in liquid cultures as well as on solid media used in the plaque-count technique. An extensive statistical treatment of results involving many replicate determinations indicated that phage multiplication could be initiated by single phage particles. Better agreement to the single particle hypothesis was obtained with young (1 d.) rather than old (5 d.) cultures. As the age of the bacterial culture increased, a smaller proportion of viable phage particles succeeded in starting phage multiplication. J. J. Jezeski

126. Bacteriophage and antibiotics affect manufacturing processes. F. E. NELSON and C. E. PARMELEE. Can. Dairy and Ice Cream J., **30,** 9: 34–35, 58. Sept., 1951.

Process difficulties arising from presence of bacteriophage and antibiotics in milk supplies constitute a growing manufacturing problem. Penicillin, streptomycin and aureomycin, which are used most extensively in treatment of mastitis, all have a definite inhibitory effect on the lactic streptococci. Heat treatment does not destroy antibiotic activity. Good tests, as yet, have not been developed for antibiotics nor have methods for discarding a number of milkings been worked out. Bacteriophage action on lactic cultures is characterized as a partial or complete cessation of acid production as the sensitive culture is lysed by the bacteriophage. Some resistant strains of bacteria may be present in a culture, or secondary organisms may develop that are resistant. The rate of increase of bacteriophage particles is extremely rapid. One strain of bacteriophage usually will act upon only a limited group of lactic streptococci. The substitution of a culture from an entirely different source frequently gives at least temporary relief. Most bacteriophage strains survive pasteurization and some strains will tolerate 158° F. for 120 min. Heating to about 190° F. for 1 hr. will destroy the bacteriophage, as will autoclaving. H. Pyenson

127. 'Streptozyme', a lytic enzyme from lactic streptococci. A. HIRSCH and D. M. WHEATER. Nature, **168,** 4275: 607. 1951.

The name Streptozyme has been applied to a lysing enzyme, produced by several strains of lactic streptococci isolated from silage. The inhibitory substance, tested against *Staph. aureus,* is inactive in acid media and is heat labile. R. Whitaker

128. Growth and nisin production of a strain of Streptococcus lactis. A. HIRSCH, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Gen. Microbiol., **5,** 1: 208–221. 1951.

A strain of *S. lactis* capable of producing high yields of nisin in milk was cultured under a variety of conditions in an attempt to increase production of the antibiotic. Continuous sub-culturing and attempts at chemically induced variation did not produce any notable changes. Nisin was

stable in culture fluids in presence of developed acid but was inactivated by heating at pH 6–9. If developed lactic acid was neutralized, growth was increased in the presence of glucose until pantothenate became limiting. Optimum yields of nisin were obtained at pH 6 in the presence of sufficient glucose and pantothenate.

J. J. Jezeski

129. The assay for the antibiotic nisin. A. HIRSCH, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Gen. Microbiol., **4,** 1: 70–83. 1950.

The properties of nisin control the type of assay possible for this antibiotic. Various assay methods were investigated, including dilution methods, bactericidal method and lag phage method. Limitations of these methods are discussed and conditions required for a valid assay procedure were stated. J. J. Jezeski

130. 'Lactobacillin', an antibiotic from lactobacilli. D. M. WHEATER, A. HIRSCH and A. T. R. MATTICK. Nature, **168,** 4276: 659. 1951.

Using *Staph. aureus* as the test organism, 40 strains of lactobacilli possessed antibiotic properties. These strains, representing about 3% of those tested, were obtained mostly from Gruyere cheese produced in different areas in France. One organism was studied in detail and best growth conditions established.

R. Whitaker

131. Studies on degradation of fats by microorganisms. I. Preliminary investigations on enzyme systems involved in the spoilage of fats. S. MUKHERJEE, Univ. of Calcutta, Calcutta, India. Arch. Biochem. Biophysics, **33,** 3: 364–376. Oct., 1951.

Twelve strains of molds (5 *Aspergillus niger,* 2 each of *Penicillium glaucum, A. flavoryzea* and *A. fumigatus,* and 1 of *P. italicum*) were isolated from local market butter; spores of all 12 strains were able to grow in sterilized butterfat containing as little as 0.5% moisture; development of ketones reached a max. after 50-d. incubation at 37° C. Water extracts of the molds were found to contain lipoxidase, peroxidase, dehydrogenase and oxidase. The mechanism of rancidification of fats embodies the initial step of hydrolysis of glycerides by lipases, followed by rapid oxidation of fatty acids by simultaneous action of fatty acid oxidases on lower saturated acids, lipoxidase, oxidation of unsaturated acids and dehydrogenase desaturation of the saturated acids, and secondary decompositions by peroxidase of peroxides formed by autoxidation and lipoxidase activity.

H. J. Peppler

132. Research on lacto-mannitic bacteria. X. Simultaneous fixation of trioses, pyruvic acid and acetaldehyde in heterolactic fermentations with living cells. V. BOLCATO, Univ. of Pavia. Enzymologia, **14,** 1: 21–23. 1950.

In the fermentation of sucrose and fructose by a heterolactic bacterium, trioses, pyruvic acid and acetaldehyde were found to be intermediate products. Phenylhydrazine oxalate was used in the fixation of these compounds during fermentation by washed living cells. J. J. Jezeski

133. Isolation of a bacterium producing propionic acid from the rumen of sheep. A. T. JOHNS, Univ. of Cambridge. J. Gen. Microbiol., **5,** 2: 317–325. 1951.

A yeast extract-sodium lactate medium was used to isolate a strictly anaerobic micrococcus from fistulated sheep. The organism was identified as *Veillonella gazogenes* and was capable of producing acetic acid, propionic acid, CO_2 and hydrogen from lactate, but was unable to ferment any of the common sugars. The fermentation reaction of this organism on lactate was studied by means of the Warburg technique and led to the finding that succinic acid was decarboxylated to produce propionic acid and CO_2.

J. J. Jezeski

134. The mechanism of propionic acid formation by Veillonella gazogenes. A. T. JOHNS, Univ. of Cambridge. J. Gen. Microbiol., **5,** 2: 326–336. 1951.

Manometric experiments utilized washed suspensions of the organism capable of producing propionic acid. The mechanism of propionic acid formation from lactate appeared to proceed from lactate to pyruvate to oxaloacetate to malate to fumarate to succinate to propionate plus CO_2. Tartrate was not attacked if the organisms were grown on lactate. Using $Na_2C^{13}O_3$, CO_2 was fixed in the carboxyl group of propionic acid during the fermentation of lactate and the amount of propionic acid produced from lactate is influenced by the CO_2 concentration in the medium.

J. J. Jezeski

135. The mechanism of propionic acid formation by propionibacteria. A. T. JOHNS, Univ. of Cambridge. J. Gen. Microbiol., **5,** 2: 337–345. 1951.

Organisms of the genus *Propionibacterium* appear to produce propionic acid mainly by the decarboxylation of succinic acid produced from either lactate or glucose. The pH of the fermentation apparently governs the ratios of propionic and acetic acids produced in these fermentations and, in addition, variations in CO_2 tension likewise may influence these ratios. Organisms used included *Propionibacterum shermanii* and *Propionibacterium zeae.* J. J. Jezeski

136. The adsorption of cetyltrimethylammonium bromide by bacteria, its action in releasing cellular constituents and its bactericidal effects. M. R. J. SALTON, Univ. of Cambridge. J. Gen. Microbiol., **5,** 2: 391–404. 1951.

The maximum amount of cetyltrimethylammonium bromide (CTAB) adsorbed by selected cultures of bacteria varies with the individual culture and the form of the uptake curve is that of an adsorption isotherm. Suspension of cells in CTAB solutions resulted in a release of substances showing maximum absorption at 260 mμ. Gram positive bacteria showed, in addition to the loss of 260 mμ-absorbing substance, a loss of free glutamic acid and inorganic P from the cells.

E. coli differed in that no loss of free glutamic acid was observed. The rate of release of cell constituents may be controlled by the temperature or concentration of CTAB used.

J. J. Jezeski

137. The serological identity of a yellow-pigmented Streptococcus. C. L. HANNAY, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Gen. Microbiol., **4,** 3: 294–297. 1950.

A yellow-pigmented *Streptococcus* closely related to *Strep. faecalis* was isolated from dairy cows. Raffinose was fermented and tyrosine was decarboxylated. The organism belonged to Lancefield group D. J. J. Jezeski

138. Note on an apparatus for the rapid dispensing of agar into test-tubes. H. W. JOHNSTON, Dept. Sci. Ind. Research, Wellington, New Zealand. New Zealand J. Sci. Technol., **32B,** 5: 26–27. Mar., 1951.

An apparatus is illustrated and described for tubing agar without wetting the tube near its lip.

W. C. Frazier

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

139. Effect of heat on milk constituents. I. A. GOULD. Can. Dairy and Ice Cream J., **30,** 9: 54, 57. Sept., 1951.

The complexity of the milk system increases with application of heat, and new techniques are required to assess the effects of heating. When milk is heated, the first major change is the denaturation and subsequent coagulation of the whey proteins. At 143° F. for 30 min., or 160° F. for 15 sec., from 5–10% of these proteins are denatured. As the temperature and heating time are increased, rapid denaturation occurs and at 176° F. for a few minutes it is essentially complete. Temperatures near boiling produce other changes, such as hydrolysis of whey proteins, increases in proteose and non-protein nitrogen and the production of ammonia. Lactose undergoes decomposition to form such acids as formic and lactic and to produce organic compounds such as furfuryl alcohol and maltol. The changes in protein and lactose affect both the color and flavor in the milk. The salts of milk constitute one of the most complex systems of the milk components. Ca appears to be the balance wheel in the salt system of milk as it relates to the effect of heat. H. Pyenson

140. La recupération et la purification du lactose au moyens des résines échangeurs d'ions (The recovery and purification of lactose by means of ion exchange resins). G. GENIN. Lait, **31,** 308: 511–518. Sept.–Oct., 1951.

The subject of ion exchange is developed historically and the mode of action of various resins and 2 methods of recovering lactose from cheddar cheese whey by use of ion exchange are reviewed. S. Patton

141. Le dosage des chlorures dans le lait et les produits laitiers par la méthode de E. Votocek (The determination of chlorides in milk and dairy products by the method of E. Votocek). L. M. BURUIANA and P. C. NICULESCO. Lait, **31,** 308: 481–487. Sept.–Oct., 1951.

Votocek's method for determining chlorides was applied to cow's milk, cheese and the milk of several other species. It was considered superior to argentometric methods. The method consists of deproteinating the medium with NaOH and $ZnSO_4$, titrating an aliquot of clear filtrate with $Hg(NO_3)_2$ solution, sodium nitroprusside being employed as an indicator. The chlorides present are bound in an indissociable form as $HgCl_2$. A sharp end point is given through reaction of excess $Hg(NO_3)_2$ with nitroprusside to form a white precipitate. A calibration table and graphs, to correct for lack of proportionality in the titration of very small quantities of chlorides, are presented. S. Patton

142. Determining fat content. H. C. HANSEN and E. D. MCGLASSON, U. of Idaho, Moscow. Ice Cream Field, **58,** 4: 94–97. Oct., 1951.

The results are given of a study in which fat determinations were made of ice cream mixes by the following methods: (a) Mojonnier test (as standard), (b) Kiaseff test, (c) Swope test, (d) butyl alcohol test, (e) Nebraska or Crowe test, (f) Overman-Garrett test, (g) Minnesota test no. 1, (h) Minnesota test no. 2 and (i) perchloric acid test.

Most of the tests using alcohol as a reagent gave results that were considerably higher than the Mojonnier results and consequently can not be considered satisfactory. Only the perchloric acid test and the Minnesota no. 1 test gave results that were in acceptable agreement with the Mojonnier results. These tests both averaged 0.013% above the Mojonnier fat tests.

W. C. Cole

143. Perchloric acid test for fat. MARIA REXACH and P. H. TRACY, U. of Ill., Urbana. Ice Cream Field, **58,** 5: 60, 62. Nov., 1951.

The authors worked out 2 modifications of the method developed by Smith, Fritz and Pyenson for determining the butterfat content of ice cream employing mixtures of perchloric and acetic acids. Procedures are given for (a) high and medium fat mixes and (b) low fat mixes. The authors stress the importance of adhering closely to these procedures if satisfactory results are expected. W. C. Cole

144. Détermination de la teneur en matière grasse des frommages au moyen du butyromètre Gerber (Determination of fat content of cheeses by means of the Gerber butyrometer). A. GRANVILLE and F. DESMET. Lait, **31,** 308: 501–505. Sept.–Oct., 1951.

The study adapted the Gerber butyrometric method to the determination of fat in cheeses. Cheese (2 g.) is weighed onto a tared piece of cellophane. The cheese is rolled like a cigarette in the cellophane and injected into the butyrometer containing 10 ml. of Gerber H_2SO_4. Water is added to make up the sample to 11 g. after which 1 ml. of amyl alcohol is added. The

sample is shaken until digested, then placed in a water bath at 65–68° C. for 15 min., during which time it is agitated frequently. Following centrifuging 10 min. and tempering in the water bath for 10 min., the test is read and the fat content derived from the formula:

$$\% \text{ fat} = \frac{\text{reading} \times 11}{\text{wt. of sample.}}$$

Comparison of results from the method with those from others was found satisfactory, except that with aged cheeses, the Gerber adaption yielded high results, presumably due to inclusion of fermentation products such as free fatty acids, which might be excluded in other methods.

S. Patton

145. Determination of small amounts of 2,4-dichlorophenoxyacetic acid in milk. R. P. Marquardt and E. N. Luce, The Dow Chemical Co., Midland, Mich. Ind. Eng. Chem., **23,** 10: 1484–1486. Oct., 1951.

Wide use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a weed killer makes it desirable to have a method for determining very small quantities of this acid in foods. The 2,4-D first is separated from the milk constituents by extraction of fat with ether and precipitation of casein by acid. Soluble proteins are removed from the casein-free filtrate by precipitation with phosphotungstic acid. The 2,4-D is extracted from the protein-free filtrate with ether and determined colorimetrically by use of chromotropic acid. Concentrations of less than 0.2 ppm. of 2,4-D (0.1 mg./pt. of milk) can be determined by this procedure. B. H. Webb

146. Water-insoluble fatty acids and butyric acid in butter manufactured by the "continuous" process. F. Hillig and S. W. Ahlmann, Food and Drug Admin., Washington, D. C. J. Assoc. Off. Agr. Chem., **34,** 4: 777–782. 1951.

Some WIA were removed during the centrifuging step in the continuous process, since the amount present in the separated oil before vacreation was less than that of the cream. Little difference was noted in the WIA content of the oil before and after vacreation and in the finished butter. Neutralization of the oil neither increased nor decreased the WIA. Butters churned from the same vat of cream by the continuous process and with a barrel churn contained the same quantity of WIA. With the continuous process, about one-third of the butyric acid present in the cream was retained in the oil. No butyric acid was lost as a result of vacreation, neutralization and churning of the oil. Most of the butyric acid present in cream was not present in butter churned by a barrel-type churn. The mold mycelia content of butter made by the continuous process was much less than that made in a barrel-type churn. F. J. Babel

147. Effect of excess alkali in the determination of water-insoluble fatty acids in butter. F. Hillig, Food and Drug Admin., Washington, D. C. J. Assoc. Off. Agr. Chem., **34,** 4: 782–787. 1951.

Ten samples of butter were tested for WIA after neutralizing the ether-water solution of the butters with *N* NaOH to (a) a decided pink color, (b) a decided pink color with 0.5 ml. *N* NaOH in excess and (c) a decided pink color with 2.0 ml. *N* NaOH in excess. Statistical treatment of the data indicated a significant difference in WIA with use of 2.0 ml. *N* NaOH in excess of that necessary to give a decided pink color. However, no significant difference resulted with neutralization to a definite pink color, and 0.5 ml. in excess of this amount. In another series of 20 butter samples, 1 sample showed 283 mg. of WIA /100 g. fat when neutralized to a faint pink color and 548 mg. when an excess of 0.5 ml. *N* NaOH was added. This sample was neutralized with lime. Further tests showed that creams neutralized with lime gave butters which were sometimes much higher in WIA when tested by the regular procedure (neutralization to a decided pink color plus 0.5 ml. *N* NaOH) than when neutralized to a faint pink color. F. J. Babel

148. An inquiry into the interpretation of the freezing points of milk obtained by the Hortvet Cryoscope and its relationship to present day market fluid milk. C. Paley and B. Tzall, Certified Labs., Inc., N. Y. City. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 81–94. 1951.

The A.O.A.C. methods give the freezing point of milk as –0.550° C. and state that this figure is sufficiently accurate that any milk showing more than 3% of added water shall be considered to be adulterated with water. The added water shall be determined by the formula:

$$\text{Added water} = \frac{T - T'}{T} \times 100,$$

in which $T = -0.550$ and T' = determined freezing point.

Tests on 1,450 fluid market milk samples showed only 0.65% of them to have freezing points lower than –0.550; 40% of the samples came within the limits set by the A.O.A.C. for pure milk. The frequency distribution curve showed a peak at –0.531° C., with 33% of all samples being between –0.530 and –0.535° C.

The question was raised concerning the accuracy of the present standard on modern market milk to indicate the amount of added water.

A. C. Dahlberg

149. The detection of enzymes by the chromatographic brush method. I. L. Zechmeister and M. Rohdewald, Calif. Inst. of Technol., Pasadena. Enzymologia, **13,** 6: 388–392. 1951.

After adsorption on columns of activated alumina, enzymes were located by brushing on various substrates along the length of the columns. After suitable short incubation periods, specific reagents capable of producing color reactions with the end products were likewise brushed on over the columns. Sites of specific enzyme activity could be located by the appearance of color. J. J. Jezeski

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

150. Vacuumizer may be answer to entrapped air problem. N. MYRICK. Am. Milk Rev., **13,** 11: 36, 84, 85. Nov., 1951.

The vacuumizer is a piece of dairy plant equipment designed to remove entrapped air and undesirable odors. Breuninger's Dairy, Phila., Pa., has the 1st commercial installation. The device is a cylinder approximately 3.5 ft. high and 2.5 ft. in diameter, installed between the short-time pasteurizer and the homogenizer inlet and handles 20,000 lb. of milk/hr. The milk is distributed at the top of the cylinder by means of a shallow pan with a perforated bottom. A vacuum of 12 in. removes the air and odors as the milk falls about 2 ft. to the bottom of the pan. Air trouble at the filler was practically eliminated. An added advantage, not supported by exhaustive tests, is lower bacteria counts, thought due to depriving bacteria of needed oxygen. The manufacturer is Chester-Jensen Co., Chester, Pa. D. J. Hankinson

151. Progress report on 3A sanitary standards for milk equipment and report of Committee on Dairy Industry Equipment. C. W. WEBER, N. Y. State Dept. Health, Albany. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 15–20. 1951.

This state committee cooperates with the 3A committees. An application has been made to copyright the 3A symbol by the International Assoc. Milk and Food Sanitarians and it looks as if this copyright may be granted. Milk sanitarians ought to encourage the installation of 3A equipment. Standards have been approved or considered for fittings, storage tanks, weigh cans and receiving tanks, homogenizers and high pressure pumps, thermometer wells for storage tanks, automotive transportation tanks, electric motors and motor attachments, tinned strainers, filters and timing of HTST pasteurizers by salt conductivity test. A. C. Dahlberg

152. Modern instrumentation for milk processing. G. F. BARNUM. Can. Dairy and Ice Cream J., **30,** 9: 36–39, 56. Sept., 1951.

A discussion of the various factors that go into selection of the proper automatic control system for various applications in the dairy industry is given. H. Pyenson

153. Liquid supply means for power washing cream separator. W. W. HARSTICK and H. O. VOGEL (assignors to International Harvester Co.). U. S. Patent 2,577,326. 6 claims. Dec. 4, 1951. Official Gaz. U. S. Pat. Office, **653,** 1: 176. 1951.

A device inserted into a self-cleaning separator bowl introduces solutions for cleaning the discs and bowl while the bowl is rotating.

R. Whitaker

154. Self-washing centrifuge. R. D. ACTON. U. S. Patent 2,575,506. 21 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 737. 1951.

Orifices are provided in the outer wall of this separator bowl to permit escape of cleaning fluids and slime. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

155. Federal pricing programs for the dairy industry. H. L. FOREST, P.M.A., U.S.D.A., Washington, D. C. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 101–110. 1951.

A satisfactory milk supply cannot always be assured by sanitary codes alone. It can be done only by a combination of proper sanitary codes and proper pricing to assure an adequate and dependable supply of safe, wholesome milk.

The problem of an adequate and dependable supply has been the basis for 2 major acts of Congress. The 1st deals with the pricing of milk for special markets, as given in the Agricultural Adjustment Act and the Agricultural Marketing Agreement Act. This provides for the pricing of milk in various markets. The 2nd deals with support prices for selected dairy products, based upon government purchases at selected price levels. These support price purchases of cheese and butter have been extensive but they have established minimum price levels for milk for manufacturing purposes. A. C. Dahlberg

156. Reducing milk waste in dairy plants. V. SCHWARZKOPF, Lathrop-Paulson Co., Chicago, Ill. Milk Dealer, **41,** 2: 51–52, 88–90. Nov., 1951.

Data are presented showing the effect of (a) methods of feeding cans to washer on milk losses, (b) recovery of drainable milk, (c) accumulative effect of progressive drainage, (d) total milk solids collected from 25 freshly dumped 8-gal. cans. It is shown that: (a) Incomplete recovery of milk from freshly dumped milk cans is a common source of waste. The volume of milk which remains in the dumped can is affected by the manner in which the cans are dumped and fed into the can washer. By providing for a slight pause over the weigh tank, milk losses are reduced substantially. (b) Nearly 50% of the drainable milk which remains in properly handled cans may be recovered at the load position. Therefore, means should be provided wherever possible to divert this milk into either the weigh tank or receiving tank for immediate use. (c) If the can washer is equipped with a sanitary extension to provide at least 21.5 sec. drainage beyond the load position, much milk can be saved. The milk zone of the extension should be made of stainless steel and of sanitary design, so that all parts can be easily cleaned. (d) Prolonged drainage, at least in warm weather, does not appear to be worthwhile, since 21.5 sec. beyond the load position recovered approximately 95% of all drainable milk, whereas doubling the drainage time saved less than 5% additional solids. Further study of this problem will be necessary to determine the effect of winter weather on the recovery of milk from the cans. (e) The recovered milk should be fully utilized, otherwise

it is still a part of the waste problem. If it is collected in a sanitary manner, it will be of the same quality as if it had drained directly into the weigh tank. The fact that the can is in motion while draining does not reduce the wholesomeness of the drained milk. C. J. Babcock

157. Plant employees go for automatic food service. FULLER ROSS. Am. Dairy Prod. Mfg. Rev., **13,** 10: 38–42. Oct., 1951.

Automatic food merchandising equipment helps to solve the problem of in-plant feeding of employees in small plants. It helps prevent accidents, reduces absenteeism, decreases labor turnover, increases production and improves morale. Automatic food service provides quality food and saves time, money and facilities. The advantages of the service are being recognized and installations are increasing. T. J. Claydon

158. Carnation simplifies its accounts receivables. H. E. OLSON, Carnation Co., Los Angeles, Cal. Am. Dairy Prod. Mfg. Rev., **13,** 10: 34–36. Oct., 1951.

At the Los Angeles office, Carnation installed Remington Rand's simplified unit invoice accounting plan. The streamlined system for accounts receivable permits highly efficient handling from 1 central office. Control is maintained over shipments from 40 condenseries and over 100 branch sales and brokerage offices throughout the country. Operation of the system is explained. T. J. Claydon

159. Preventing grievances. C. H. BROADED, Fisher Flouring Mills Co., Seattle, Wash. Am. Dairy Prod. Mfg. Rev., **13,** 11: 22, 23, 37. Nov., 1951.

The successful handling of employee grievances is an important problem in business organizations. A technique which has proven successful is summarized as follows: (a) locate the grievance, (b) talk with the employee, (c) get all the facts, (d) take appropriate action, (e) convince the employee of the fairness of the action. Each step is discussed briefly. T. J. Claydon

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

160. Synthesis of sulfur amino acids from inorganic sulfate by ruminants. II. Synthesis of cystine and methionine from sodium sulfate by the goat and by the microorganisms of the rumen of the ewe. R. J. BLOTK, J. A. STEKOL and J. K. LOOSLI, N. Y. Medical College, New York 29, Inst. for Cancer Research, Philadelphia 11, Pa., and Cornell Univ., Ithaca, N. Y. Arch. Biochem. Biophysics, **33,** 3: 353–363. Oct., 1951.

Within 3 hr. after ingestion of $Na_2S^{35}O_4$ by a goat, appreciable quantities of radioactive cystine and methionine were found in the milk proteins, the peak activity being reached within 24 hr. of feeding. In the proteins of milk, serum albumin and rumen contents the radioactivity was observed to be proportional to the amount of each sulfur-containing amino acid in the protein, suggesting that cystine and methionine were synthesized in the rumen at an equal rate and were used by the tissues to form new protein in the quantities needed. H. J. Peppler

161. The conversion of certain soluble sugars to a glucosan by holotrich ciliates in the rumen of sheep. A. E. OXFORD, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., **5,** 1: 83–90. 1951.

Strained rumen contents from hay-fed sheep serving as the source of protozoa were inoculated into small fermentation flasks containing buffered solutions of glucose, fructose or sucrose. Microscopic granules of a glucosan giving a purple color with iodine were observed in the holotrichously flagellated protozoa and were liberated by bursting the cells with a synthetic detergent under mild conditions. Other simple sugars were not converted into the polysaccharide granules by protozoa during *in vitro* fermentation. A similar substance could be isolated from protozoa taken directly from the rumen itself from 2–4 hr. after feeding. J. J. Jezeski

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

162. The pen type barn and its future. G. HOPSON, DeLaval Separator Co., N. Y. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 67–80. 1951.

During the past 45 yr. labor requirements in caring for dairy cows have been reduced only 10–15%. In the Northeast milk shed the majority of present dairy barns was constructed about 50 yr. ago. There needs to be radical changes to reduce labor requirements.

The pen stable may reduce cost of construction by 50%. When used with the "cow cafeteria" feeding system, much labor is saved. The combine or pipeline system of milking is the greatest advance in milking ever offered to the dairymen. By utilizing these 3 features much labor can be saved.

The pen stabling requirements of the New York State Dept. of Health are presented. It is realized that some sanitarians have misgivings about cows sleeping on the manure pile, considering the years of effort to get dairymen to remove manure daily from the barns and yards. The quality of milk can be excellent under good management and milking practices. A. C. Dahlberg

163. Removal of air from dairy stables. W. KALBFLEISCH and J. W. WHITE, Cent. Exptl. Farm, Ottawa. Sci. Agr., **31,** 11: 492–495. Nov., 1951.

Under extreme winter conditions, removal of moisture from dairy stables is a problem because of the difficulty of doing so without chilling the stable. Results of a study in which the heat losses were compared when floor and ceiling ventilators were used are presented. Heat losses/100 grains of water removed were less with ceiling ventilators. Outside temperatures ranged from −9 to −30° F. O. R. Irvine

164. Adjustable comfort cow stall. C. SCHILLINGER. U. S. Patent 2,578,093. 3 claims. Dec. 11, 1951. Official Gaz. U. S. Pat. Office, **653,** 2: 438. 1951.

A stanchion is described in which the cow's head is inserted between an inverted U-shaped yoke and an inverted V-shaped frame, the lower piece being adjustable to the height of the cow's neck. R. Whitaker

165. Control device for milking machines. A. G. PERKINS. U. S. Patent 2,576,808. 10 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1154. 1951.

An electrically operated control discontinues the milking operation when flow of milk from the cow ceases. R. Whitaker

166. Milking machine trap. W. H. HARSTICK. U. S. Patent 2,581,530. 7 claims. Jan. 8, 1952. Official Gaz. U .S. Pat. Office, **654,** 2: 450. 1952.

A device is designed to be installed on the top of the milk-collecting receptacle to allow discharge of milk from the suction line of a milking machine into the receptacle. R. Whitaker

167. Calf weaner. H. F. VANCIK. U. S. Patent 2,575, 433. 5 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 718. 1951.

A device for weaning calves consists of a wire bent in a form to fit into the nostrils and with a loop extending in front of the mouth.

R. Whitaker

168. Electric heater for stock watering tanks. J. T. LANDGRAF (assignor to H. D. Hudson Mfg. Co.). U. S. Patent 2,576,688. 8 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1125. 1951.

An L-shaped rigid electrical heating coil thermostatically controlled for warming the water in cattle watering tanks is described. R. Whitaker

169. Cattle restraining apparatus. A. E. THORSON. U. S. Patent 2,576,654. 3 claims. Nov. 27, 1951. Official Gaz. U. S. Pat. Office, **652,** 4: 1116. 1951.

A device for restraining cattle to permit branding, operating, clipping, etc., is described.

R. Whitaker

170. Dehorning paste applier with magazine feed. B. L. GOLDEN and T. J. SULLIVAN. U. S. Patent 2,580,169. 9 claims. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1134. 1951.

A plunger-type tool for scraping the base of the horns of cattle and applying horn-inhibiting material to the prepared area is described.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

171. Low lactose solids. T. A. NICKERSON, U. of Calif., Davis. Ice Cream Field, **58,** 4: 90–91. Oct., 1951.

Results are given of experiments with ice cream in which normal milk-solids-not-fat are compared with low-lactose milk-solids-not-fat with respect to whipping properties of mixes and tendency for sandiness to occur in ice cream during storage.

When De Lac powder (a low-lactose milk powder) was used in place of normal milk-solids-not-fat in ice cream mixes it was observed that there was an improvement in whipping properties of the mixes during freezing. The low-lactose ice cream showed less tendency to develop sandiness than mixes of similar milk-solids-not-fat made from normal sources.

It is concluded that "there is a place in the ice cream industry for milk products modified in composition or with special properties to minimize specific ice cream problems." W. C. Cole

172. Do we need to revaluate ice cream composition? P. H. TRACY, Univ. of Ill., Urbana. Ice Cream Trade J., **47,** 12: 22, 89. Dec., 1951.

At present 4 states and the District of Columbia have an 8% fat standard, 25 states have a 10% standard, 15 states have a 12% standard, 1 has a 13% standard and 3 have a 14% standard. Today commercial ice cream contains about 12–14% fat, 10–11% solids-not-fat and 15% sugar. Farm pressure groups have tried to keep the fat standard high on the assumption that it will create a greater demand for their products. Some manufacturers have made high-fat ice cream for competitive reasons. In recent years the per capita consumption of ice cream has been declining from 4.9 gal. in 1946 to 3.4 gal. in 1951. The ice cream industry is vulnerable to a decrease in consumer purchasing power and the industry may suffer if the consumer's purchasing power decreases. Manufacturers of ice cream find their costs increasing, making it necessary to pass this along to the consumer by increasing prices. The problem that faces the industry today is to bring ice cream back to within the reach of the middle- and low-income groups. If the industry wants to hold its own against the competitive forces of the other food industries, it may be desirable to consider the manufacture of frozen products which contain less fat and put more emphasis on the serum solids. W. H. Martin

173. Stabilizer study. W. A. KRIENKE, Florida Agr. Expt. Sta., Gainesville. Ice Cream Field, **58,** 4: 98, 99, 110. Oct., 1951.

This reports preliminary experiments with several stablizers used in ice cream. Mix whipability and viscosity, as well as body and texture of the ice cream were considered in comparing the following ice cream stabilizers used in the concentrations indicated: 0.15% sodium carboxymethyl-cellulose, 0.15% sodium cellulose sulfate, 0.05% Irish moss, 0.25% sodium alginate and 0.35% gelatin.

Sodium cellulose sulfate and gelatin gave mixes of the lowest viscosity. All except the gelatin mix gave better whipping properties than the control mix with no stabilizer when frozen in a batch freezer. All of the stabilizer samples showed improved body and texture as compared with the no-stabilizer control. It is concluded that sodium cellulose sulfate and domestic "Irish" moss col-

loid warrant further consideration as ice cream stabilizers. W. C. Cole

174. The effect on mix of HTST pasteurization. W. S. Arbuckle and J. W. Nisonger, U. of Maryland, College Park. Ice Cream Field, **58,** 6: 60, 61, 68. Dec., 1951.

Experimental mixes were pasteurized by the HTST method by holding them at 170, 180, 190 and 200° F., respectively, for 15 sec. After processing, the mixes were aged 18 hr. and then frozen in both batch and continuous freezers.

There was a tendency for mix viscosity to decrease as pasteurization temperature increased but the body and texture characteristics of the ice cream were but little affected by pasteurization temperature. Mixes pasteurized at 190 and 200° F. did not develop off-flavors during a 6-mo. storage period, whereas those pasteurized at 170 and 180° F. did develop off-flavors.

W. C. Cole

175. High-temperature short-time pasteurization above 160° F. in vacuum. F. W. Barber and H. P. Hodes. Natl. Dairy Research Lab., Oakdale, N. Y. 24th (1950) Ann. Rpt., N .Y. State Assoc. Milk Sanit., 173–186. 1951.

The Vacreator has been used for the pasteurization of cream for butter-making, milk for cheese making and of ice cream mix. A further study of last process was made. A test organism, *Micrococcus* MS102, was used to give a count of 500,000–1,000,000 in the mix.

The heat-resistant test organism is destroyed at 175° F. for 25 sec., and at 190° F. for 1.4 sec. in the Vacreator. This compared with 155° F. for 30 min. in laboratory vat equipment. Also these 3 heat treatments gave similar results for destruction of the usual bacteria in the mix. The body, texture and flavor of ice cream were satisfactory.

A. C. Dahlberg

176. Agitating and scraping mechanism. T. Carvel. U. S. Patent 2,576,995. 22 claims. Dec. 4, 1951. Official Gaz. U. S. Pat. Office, **653,** 1: 90. 1951.

Details are given for a freezer for continuously producing ice cream and other frozen confections.

R. Whitaker

177. Ice cream freezer. M. A. Rollman. U. S. Patent 2,577,916. 11 claims. Dec. 11, 1951. Official Gaz. U. S. Pat. Office, **653,** 2: 390. 1951.

A design is given for an ice and salt vertical-type ice cream freezer, with the sides of the freezing chamber slightly tapered and the dasher powered by a shaft entering the freezer from below.

R. Whitaker

178. Stabilization in storage of fruit for ice cream. C. Koerver. Can. Dairy and Ice Cream J., **30,** 10: 39–42, 58. Oct., 1951.

Frozen fruits require careful treatment and storage to develop their best characteristics in ice cream. The fruits discussed are strawberries, peaches, raspberries, pineapples and cherries. The main job of the cold packing plant is to safeguard perishable fruit quickly in as near its original condition as possible and to be able to store it for future use. The addition of stabilizers to fruit packs has not gone beyond the experimental stage and is far from general acceptance.

H. Pyenson

179. Method of making variegated ice cream. E. C. Lehner (assignor to Swift & Co.). U. S. Patent 2,576,842. 10 claims. Nov. 27, 1951. Official Gaz. U .S. Pat. Office, **652,** 4: 1162. 1951.

Flavoring material, liquid at warm temperatures but solid at sub-freezing, is injected into semi-frozen ice cream, where it forms irregular-shaped pieces. The flavored ice cream then is passed through another freezer to complete the freezing operation and thoroughly distribute flavoring material. R. Whitaker

180. Black walnut oil fortifies ice cream's flavor. C. C. Flora, L. L. Davis and C. W. Holdaway, Virginia Agr. Expt. Sta., Blacksburg. Ice Cream Field, **58,** 4. 92, 105. Oct., 1951.

Previously published results showed it advantageous to use fruit extracts along with fruit in making various fruit ice cream. A report is given of experimental results in which oil expressed from black walnut screenings was added to mixes used in making black walnut ice cream.

Black walnut oil added at the rate of 200–400 ml./45 lb. of mix markedly improved the flavor of the ice cream. Homogenization of the mix and oil increased the flavor intensity, as compared to adding the oil without homogenization. Ice cream fortified with black walnut oil, but without black walnut granules, was preferred to ice cream with granules alone. W. C. Cole

181. Holder for ice cream cans. W. C. Kuykendall. U. S. Patent 2,575,592. 2 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 760. 1951.

Cans are held in place and prevented from turning in the sleeves of ice cream cabinets by metal rings and inflated bags. R. Whitaker

182. Standardizing the half-gallon rectangular container. A. H. Bayer, Gen. Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., **47,** 12: 72, 74, 92. Dec., 1951.

An effort is being made by the Simplified Practice Committee of the International Association of Ice Cream Manufacturers to standardize the 0.5-gal., rectangular ice cream container. A study revealed that there were at least 6 different containers on the market whose calculated cubical content varied from 111.58–116.25 in.^{3}, whereas the actual calculated cubical content of the 0.5-gal. container is 115.5 in.^{3}

The committee developed special equipment for measuring the actual distortion resulting from assembling a carton empty, the additional distortion developed when filled with soft ice cream from the freezer and then the additional distortion after hardening. An attempt is being made to develop a standardized carton.

W. H. Martin

183. Frozen confection apparatus. D. L. PERLMAN. U. S. Patent 2,579,696. 6 claims. Dec., 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1009. 1951.

An apparatus for layering different flavored ice cream and other frozen confections in horizontal circular zones in containers is described.

R. Whitaker

184. Plastic mold for ice cream, confections, etc. J. KAPPEL. U. S. Patent 2,578,361. 2 claims. Dec. 11, 1951. Official Gaz. U. S. Pat. Office, **653,** 2: 509. 1951.

A design for a mold for making fancy forms is given. R. Whitaker

185. Defrosting apparatus for freezing molds. M. KLEIN (assignor to Eskimo Pie Corp.). U. S. Patent 2,579,931. 2 claims. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1072. 1951.

Frozen materials are removed from molds by momentary heating with gases introduced into jackets adjacent to the mold pockets.

R. Whitaker

186. Mold for forming a precut ice cream cake. F. ADAMS. U. S. Patent 2,579,640. 1 claim. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 994. 1951.

A round pan with segmented chambers is designed to be filled with semi-frozen ice cream. After hardening, the pie-shaped pieces are removed by being impaled on pins set in the circular plate making up the cover. R. Whitaker

187. Device for moving ice cream cones into position for receiving ice cream and then delivering them to a customer. A. J. TACCHELLA (assignor to Steady-Flow Freezer Co.). U. S. Patent 2,580,257. 14 claims. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1158. 1951.

A machine is described which automatically fills cones with soft ice cream from a constantly maintained supply of product. R. Whitaker

188. Machine for packaging ice cream and similar frozen food products. H. R. SCHULTZ (assignor to Redi Products Corp.). U. S. Patent 2,579,096. 3 claims. Dec. 18, 1951. Official Gaz. U. S. Pat. Office, **653,** 3: 765. 1951.

Ice cream, taken directly from a continuous freezer, is extruded into packages placed in position automatically by this machine. When the prescribed amount of product has been filled into the positioned container, a cutting device severs the stream of extruded ice cream.

R. Whitaker

189. Automatic outlets. ANONYMOUS. Ice Cream Field, **58,** 3: 24. Sept., 1951.

The sale of packaged ice cream through automatic vending machines is relatively new, but it is claimed that it can do for the ice cream industry what the automatic vender did 10 yr. ago for candy, gum and soft drinks. Industrial plants and military camps are considered the best locations for these automatic venders, since they help answer the problem of in-plant feeding with a minimum of time and expense. W. C. Cole

190. Here today—Here to stay. E. C. BRUNST, JR., The Kroger Co., Cincinnati, O. Ice Cream Field, **58,** 3: 39–40. Sept., 1951.

The use of self selection display ice cream cabinets in super markets is considered an innovation that is here to stay. Cabinet location within the store is considered an important factor for successful sales.

The author predicts: (a) There will be no appreciable seasonal fluctuations of ice cream sales by the food retailer. (b) Improved refrigerated merchandising equipment will be developed to increase impulse buying. (c) Food department stores will handle 2 or more demand brands of ice cream. (d) Larger unit sales (0.5-gal., 3-qt. and gal. units) will replace present preponderance of sales in pints. (e) Ice cream insulated bags may be eliminated with the development of special insulated cartons. W. C. Cole

191. Insulated packages. ANONYMOUS. Ice Cream Field, **58,** 2: 16–18, 20. Aug., 1951.

Factory-insulated ice cream packages are now being used by such concerns as H. P. Hood & Sons, the Borden Co., Bowman Dairy Co. and others.

The procedure used in 1 plant is given. Plain white paraffined cartons are filled with ice cream and quick-hardened. Except for a notation as to flavor, this linerless package is unprinted. The package of hardened ice cream is next slipped into a sleeve-type corrugated insulator and then overwrapped with waxed paper, carrying brand name, flavor of product and advertising message. The insulated sleeve may be preformed or die-cut in the plant. Coding similar to that used by the frozen food industry is used in some plants.

This type of packaged ice cream now is being tested in various localities. Several makes of equipment suitable for insulated packaging operations are now available. W. C. Cole

192. Home storage capacity for ice cream. V. M. RABUFFO. Ice Cream Trade J., **47,** 12: 22, 100. Dec., 1951.

Twenty-four million refrigerators and 3,400,000 home freezers have been sold since 1946 making it possible for millions of homes to have ice cream available at all times. The most popular sizes in home freezers are those with 11–16.9 ft.^{3} capacity.

W. H. Martin

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

193. A study to determine the optimum temperature and point in process for the clarification of market milk. B. J. DEMOTT, H. C. HANSEN, E. D. MCGLASSON and J. C. BOYD, Univ. of Idaho, Moscow. Milk Dealer, **41,** 3: 48, 83, 85. Dec., 1951.

The more milk is heated and agitated, the more the extraneous matter will dissolve into the milk. Variations in clarification temperatures have no effect on the number of leucocytes removed and make no difference in the percentage of bacteria killed in subsequent pasteurization. Cream volume on the pasteurized sample is

affected most if the clarification is done at 135° F. and affected about equally when clarified at 40–50° F. or at 143° F. after pasteurization. Optimum temperature for clarification of market milk is 40° F. or lower, preferably before any agitation or heat is applied to the milk.

C. J. Babcock

194. Sediment control in homogenized milk. W. L. DUNKLEY and H. O. SMITH, Univ. of Cal., Davis. Milk Dealer, **41,** 2: 47–48, 107–110. Nov., 1951.

Results indicate that there is not a close relation between the cell count and sediment in homogenized milk. This was the case with milk from individual cows or pairs of cows homogenized without clarification and with mixed herd milk subjected to different clarification treatments. It was concluded that cell counts are of little value as a criterion of the effectiveness of sediment prevention. Storing homogenized milk in square qt. bottles placed on a rack which holds them on a corner at 45° angle provides a simple measure of sediment. Results obtained confirm previous reports that clarification after homogenization, rather than before, is more effective in reducing sediment. However, clarification at any point in the process will normally give satisfactory control of sediment; best results may be expected when milk is clarified hot following homogenization. Where clarification before homogenization is more convenient, cold clarification is preferable. Data are presented showing (a) cell count and Hotis tests on original milk and sediment ratings and color of sediment in homogenized milk from individual cows or pairs of cows, (b) influence of different clarifying conditions on sediment rating and cell removal and (c) sediment ratings and cell removal from homogenized milk processed with different clarification procedures in a commercial market milk plant.

C. J. Babcock

195. Preservation of milk by radiation. E. L. GADEN, JR., E. J. HENLEY and V. P. COLLINS, Columbia Univ., New York. Food Tech., **5,** 12: 506–509. 1951.

Samples of unpasteurized milk obtained from a processing plant were treated with 2 m.e.v. X-rays at dosages up to 1,830,000 Roentgen (R). Dosages up to 100,000 R. had no effect on the flavor of the milk, but at higher levels of irradiation the milk acquired an undefined off-flavor. A dosage of 146,000 R. was required to destroy 99.5% of the microorganisms detectable by the standard plate count. Samples receiving dosages of 146,000 R. or above remained salably fresh at 10° C. for periods in excess of 28 d. Irradiation levels of 146,000 or less had no apparent effect on vitamin A and riboflavin content, but a dosage of 300,000 R. caused a 40% decrease in riboflavin and slight destruction of vitamin A.

E. R. Garrison

196. Milk temperature conditioning vessel. M. A. SODEN. U. S. Patent 2,576,050. 7 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 881. 1951.

A jacketed tank with a built-in trough around the top of the tank is designed so that milk flowing into the tank forms a film as it runs down the wall and is heated or cooled, depending on the temperature of the circulating medium. A propeller-type agitator is suspended from the top.

R. Whitaker

197. Milk bottle holder. E. M. NAVARRO. U. S. Patent 2,575,971. 1 claim. Nov. 20, 1951. Official Gaz. U. S. Pat. Office **652,** 3: 861. 1951.

A device for locking glass milk bottles prevents theft during the interval between delivery on the door step and removal by customer.

R. Whitaker

198. Refrigerated milk delivery vehicle. P. KROHNERT. U. S. Patent 2,581,867. 5 claims. Jan. 8, 1952. Official Gaz. U. S. Pat. Office, **654,** 2: 540. 1952.

A design for a milk delivery vehicle having refrigerated storage space and unrefrigerated space for empty cases is described. R. Whitaker

199. Refrigerated milk delivery truck. C. NIELSEN (assignor to Arden Farms Co.). U. S. Patent 2,574,585. 4 claims. Nov. 13, 1951. Official Gaz. U. S. Pat. Office, **652,** 2: 420. 1951.

A refrigerated truck for delivering milk and other dairy products, with an inclined service door opening into the back of the driver's compartment, is described. R. Whitaker

200. Bottle carrying means. E. L. BLOOMQUIST. U. S. Patent 2,575,612. 4 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 764. 1951.

In this 12-bottle case, L-shaped dividers keep milk bottles in place. R. Whitaker

201. Container and bottle supporting rack. H. R. E. HEIDE (assignor to H. Hildebrandt). U. S. Patent 2,581,019. 1 claim. Jan. 1, 1952. Official Gaz. U. S. Pat. Office, **654,** 1: 235. 1952.

A rack for holding glass and paper milk bottles is described. R. Whitaker

202. Refrigerating cabinet for milk samples. S. CONKLIN. U. S. Patent 2,575,796. 11 claims. Nov. 20, 1951. Official Gaz. U. S. Pat. Office, **652,** 3: 814. 1951.

A refrigerated cabinet for storing composite milk samples is described. R. Whitaker

203. Cream whipper. G. M. LARSON (assignor to K. H. Roy). U. S. Patent 2,576,947. 2 claims. Dec. 4, 1951. Official Gaz. U. S. Pat. Office, **653,** 1: 77. 1951.

A reusable container is described for holding cream under gas pressure and provided with a valve controlled discharge spout for dispensing whipped cream. R. Whitaker

204. Dispenser for pressurized whipped cream. P. J. NIELSEN. U. S. Patent 2,580,188. 6 claims. Dec. 25, 1951. Official Gaz. U. S. Pat. Office, **653,** 4: 1140. 1951.

A device for dispensing gas-whipped cream from a pressurized container is described.

R. Whitaker

205. De Moedermelk-Centrale (The Human Milk Bank). G. G. A. MASTENBROEK. Netherlands Milk and Dairy J., **5:** 229–237. 1951.

The difference in composition of human and cows' milk and the importance of an adequate supply of human milk are discussed. At the collection centers human milk is frozen and sent to the central laboratory of the Netherlands Red Cross Society. After testing, the milk is pasteurized at 67° C. for 30 min., quickly frozen and water evaporated by means of lyophylic drying. The powder proved to be easily soluble in water and could be kept in moisture-proof bottles for years without becoming rancid. In extensive experiments with babies, a solution of the powder in water compared with fresh human milk showed no differences. W. C. van der Zant

MILK SECRETION

V. R. SMITH, SECTION EDITOR

206. Studies on milk ejection in the dairy cow. The effect of stimulus on the release of the "milk-ejection" hormone. W. G. WHITTLESTON, Ruakura Animal Research Sta., Dept. Agr., Hamilton, New Zealand. New Zealand J. Sci. Technol., **32A,** 5: 1–20. Feb., 1951.

Evidence indicates that on stimulation a 2nd lot of milk-ejection hormone may be secreted by cows that have let down only part of their milk during the first milking after the first secretion of hormone. W. C. Frazier

207. Für und wider die Zweiphasentheorie der Milchbildung (The pro and con of the two-phase theory of milk secretion). English summary. G. Eggers. Milchwissenschaft, **5,** 11: 390–391. Nov., 1950.

A short review on the above subject.
I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

208. Riboflavin content of New Zealand milks. N. JEAN CLEMOW, Otago Univ., Dunedin, New Zealand. New Zealand J. Sci. Technol., **32A,** 6: 14–17. Apr., 1951.

The average figure for New Zealand raw milk was 2γ of riboflavin/g. There was slightly less riboflavin after pasteurization of the milk.
W. C. Frazier

209. Koemelk als zuigelingenvoedsel. Kritische opmerkingen over gehomogeniseerde ("Soft Curd") milk. (Cow's Milk as a Baby Food. Critical Remarks on Homogenized ("Soft Curd") Milk). H. A. WEYERS. Netherlands Milk and Dairy J., **5:** 194–200. 1951.

In the Netherlands, boiled cows' milk commonly is used in the diet of the baby during the 1st 6 mo. A survey is given of the factors that determine the condition of the curd of cows' milk. Methods such as dilution, heat treatment, addition of acid, removal of Ca, treatment with proteolytic enzymes and increasing the number of fat globules, are described for changing the hard curd to a soft one. The growth of babies fed boiled cows' milk was practically the same as that of babies fed human milk. Cows' milk for babies usually is boiled for 3–5 min., which renders its curd sufficiently soft to make homogenizing unnecessary. W. C. van der Zant

210. Chocolate milk—a review of its nutritional value and its effect on milk consumption. ANONYMOUS. Milk Dealer, **41,** 2: 43, 72, 74. Nov., 1951.

Results of experiments designed to test the effect of cocoa upon the possible use of Ca and protein conducted at the University of Illinois and studies conducted at the Universities of Wisconsin and Chicago on the per capita milk consumption of families, students and factory workers show that chocolate milk is a nutritious food, containing milk nutrients in a concentration equal to or approximating that of the unflavored milk from which it is made. Judging from the results of human studies with high levels of cocoa in the diet, there is no cause for concern that cocoa present in chocolate milk will interfere with the body's use of milk nutrients. When chocolate milk is available the trend is toward increased total milk consumption. C. J. Babcock

211. Chocolate milk. ANONYMOUS. Milk Dealer, **41,** 3: 42, 98–100. Dec., 1951.

Chocolate milk is a food of high nutritive value. It is an excellent source of Ca, P, protein and most of the vitamins. There is considerable evidence that essential nutrients present in milk are more readily utilized from this source than from many other sources. There is no evidence that the presence of chocolate flavoring reduces the availability to humans beings of any of these milk nutrients. Experimental evidence obtained from adult humans consuming large quantities of cocoa indicates that the presence of chocolate flavoring does not result in decreased utilization of Ca and P of milk. There is no evidence from experiments on humans which indicates that nutrients of milk are less well digested in the presence of chocolate flavoring. Sugar present in chocolate milk contributes to the energy value of the milk. The milk, as commonly consumed, should produce no harmful effect on adults or children. As cocoa powder or chocolate syrup added to milk inhibits growth of bacteria likely to be found in milk, their addition after pasteurization offers little danger of contamination. The process of combining the ingredients through heating, in itself, produces a pasteurizing action. As an added precaution, most dairies pasteurize their chocolate milk after the mixing of the ingredients.
C. J. Babcock

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

212. Process for producing synthetic thyroprotein. C. W. TURNER and EZRA P. REINEKE (assignors to American Dairies, Inc., and The Quaker Oats Co.). U. S. Patent 23,429 (reissue).

11 claims. Nov. 13, 1951. Official Gaz. U. S. Pat. Office, **652,** 2: 385. 1951.

A synthetic thyroprotein is made by iodinating a protein containing tyrosine such as casein, at a pH 6.8–10 and 15–70° C. This is followed by incubation at 50–100° C. for 2–72 hr. using a catalyst of a manganese salt, such as the oxide, dioxide or sulphate and a mild oxidizing agent, such as H_2O_2 or an organic peroxide.

R.Whitaker

213. The mode of action of thyroxin. C. MARTINS and B. HESS, Physiol.-chem. Institut, Tübingen, Germany. Arch Biochem. Biophysics, **33,** 3: 486–487. Oct., 1951.

Liver mitochondria of rats injected with 4–12 mg. thyroxin over a period of 24–72 hr. exhibited lower rates of aerobic phosphorylation (av. esterified phosphate, 4.9%) than preparations from untreated rats (av. esterified phosphate, 19.2%). Thyroxin added directly to the reaction mixture containing normal mitochondria lowered the esterification values only when mitochondria were exposed to thyroxin for 30 min. at 0° C. before testing. The uncoupling action of thyroxin on oxidative phosphorylation is the primary effect on the basal metabolism rate; its other effects are considered as secondary responses to this change. H. J. Peppler.

214. Crystalline beef liver catalase: a simplified method of preparation. W. MOSIMANN, Cornell Univ., Ithaca, N. Y. Arch. Biochem. Biophysics, **33,** 3: 487–488. Oct., 1951.

Beef liver catalase was crystallized from the filtrate of fresh liver extracts treated with a cold soln. of chloroform and alcohol (1:2). Further purification was accomplished by dissolving the crystals in a minimal quantity of water, removing a white amorphous impurity by centrifugation and chilling the remaining soln. to crystallize catalase. H. J. Peppler

215. Salivary peroxidase. W. MOSIMANN and J. B. SUMNER, Cornell Univ., Ithaca, N. Y. Arch. Biochem. Biophysics, **33,** 3: 487. Oct., 1951.

A strong test for peroxidase is reported for the saliva of sheep, cow, dog, cat, horse and man. Tenfold conc. of salivary peroxidase was achieved by $(NH_4)_2SO_4$ and alcohol fractionation.

H. J. Peppler

216. On the proteolytic enzymes of animal tissues. IX. Calf thymus tripeptidase. D. ELLIS and J. S. FRUTON, Yale Univ., New Haven, Conn. J. Biol. Chem., **191,** 1: 153–159. July, 1951.

A peptidase specific for the hydrolysis of glycylglycylglycine, L-alanylglycylglycine and L-leucylglycylglycine was purified by tractionation of extracts of calf thymus with ethanol and Zn ions followed by $(NH_4)_2SO_4$ precipitation. Extracts of comminuted frozen calf thymus possessed greater tripeptidase activity than those obtained from minced fresh thymus.

H. J. Peppler

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

217. Application of chemical germicides in the dairy industry. C. K. JOHNS. Can. Dairy and Ice Cream J., **30,** 9: 29–31, 57. Sept., 1951.

This paper brings together for ready reference the basic principles and relevant facts important to efficient use of chemical germicides used to destroy microorganisms. The material is discussed under the three main headings of (a) selection of suitable product; (b) methods of using germicidal solutions; and (c) aids to better results. H. Pyenson

218. Cleaning and sanitizing permanent pipeline installations. W. H. HASKELL, Klenzade Prod., Inc., Beloit, Wis. Milk Dealer, **41,** 3: 44–45, 60–64. Dec., 1951.

The following factors which are of prime importance in the flush washing of pipelines are discussed: (a) Construction of line. (b) Concentrations of chemical cleaning compounds. (c) Temperatures of cleansing and sanitizing solutions. (d) Time involved in circulating for cleaning. (e) Application of friction. It is stated that if pipe lines are properly constructed and essential cleaning methods are applied, experience has clearly demonstrated that such lines may be cleaned in place. C. J. Babcock

219. Glass pipe in the fluid milk industry. F. F. FLEISCHMAN, JR., J. C. WHITE and R. F. HOLLAND. Cornell Univ., Ithaca, N. Y. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 187–195. 1951.

Pyrex glass tubing was installed in the milk plant at Cornell Univ. and in a milk plant in Geneva, N. Y. on the basis of in-place washing and subjection to bactericidal treatment. Cleanliness of glass pipes could be observed visually, degree of sterility was determined by the swab test and the effect on bacterial content of milk passing through the pipe line.

The circulating system of cleaning was used, consisting of flushing with cold water followed by circulating alkaline cleaning solution at 130–140° F. for 5–10 min. Bactericidal treatment consisted of circulating water at 180° F. or a chlorine solution of 200 ppm. for 5–10 min. The glass piping was maintained in a satisfactory state of cleanliness and sterility.

A. C. Dahlberg

220. The sanitary aspects of food vending machines. E. LUDEWIG, N. Y. City Health Dept. 24th (1950) Ann. Rpt., N. Y. State Assoc. Milk Sanit., 165–172. 1951.

Vending machines have almost unlimited sales possibilities. It is estimated that at least 1 billion dollars of merchandise are sold yearly in these machines.

The Sanitary Code of New York City does not mention vending machines but they come under the general scope of food distribution. The department has enumerated 15 points in sanitation which must be met in food vending machines.

Experience has shown these machines to be difficult to maintain in a sanitary condition. The problem is complicated by heavy complicated construction of vending machines and by necessity to service them wherever located. These machines are now in the milk industry and if given proper sanitary care they will be a significant factor in increasing milk sales. A. C. Dahlberg

221. Some interrelationships between public health and price regulations in the New York market. E. E. VIAL, Milk Dealers Assoc. of Metrop. New York. 24th (1950) Ann Rpt., N. Y. State Assoc. Milk Sanit., 111–128. 1951.

The effectiveness of pricing milk may depend almost entirely on health regulations.

Until 1949, the New York City Board of Health required that cream for ice cream had to come from plants it inspected. The market order was used to set a high price to producers on this cream. Ice cream plants used a minimum of this cream so sales decreased. In 1949 the Sanitary Code was changed to permit importation of western cream. Hearings then were held to reduce the price of cream for ice cream and more New York milk was used for this trade.

New York has a closed fluid cream market and Boston has an open one, due to sanitary health regulations. Before price regulations New York cream was $2.60/40-qt. can over Boston cream. With New York state price regulations this price differential remained, but under Federal-state price control the price spread increased to $8.17/can. In New York City the consumption of fluid cream decreased at a time milk consumption increased and the low cream consumption has continued.

The dating of milk bottle caps in New York City is required in the sanitary code but it increases costs, especially by returned milk, and is not required in most markets. A. C. Dahlberg

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Sample of book citation: (1) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.

For Experiment Station publications, the citation should be as follows: (1) COULTER, S. T., AND JENNESS, R. Packing Dry Whole Milk in Inert Gas. Minn. Agr. Expt. Sta. Tech. Bull. 167. 1945.

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