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Contents

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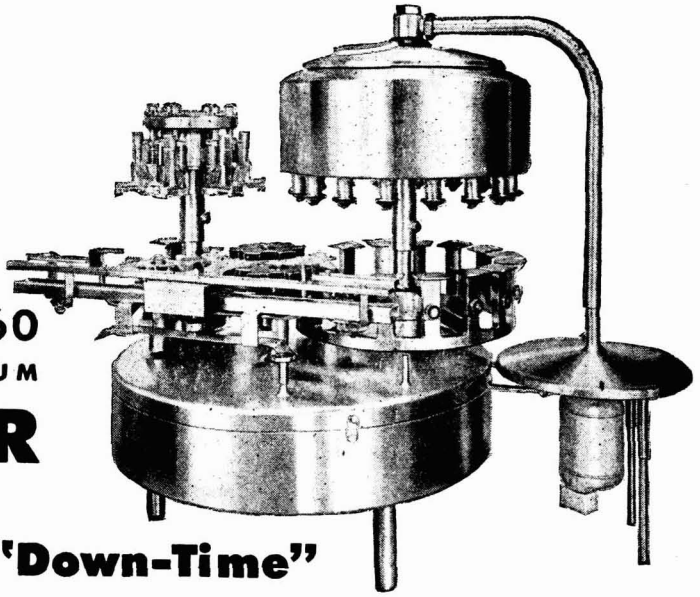

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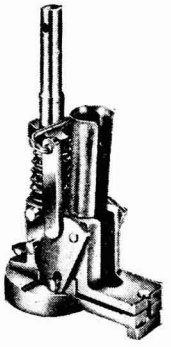

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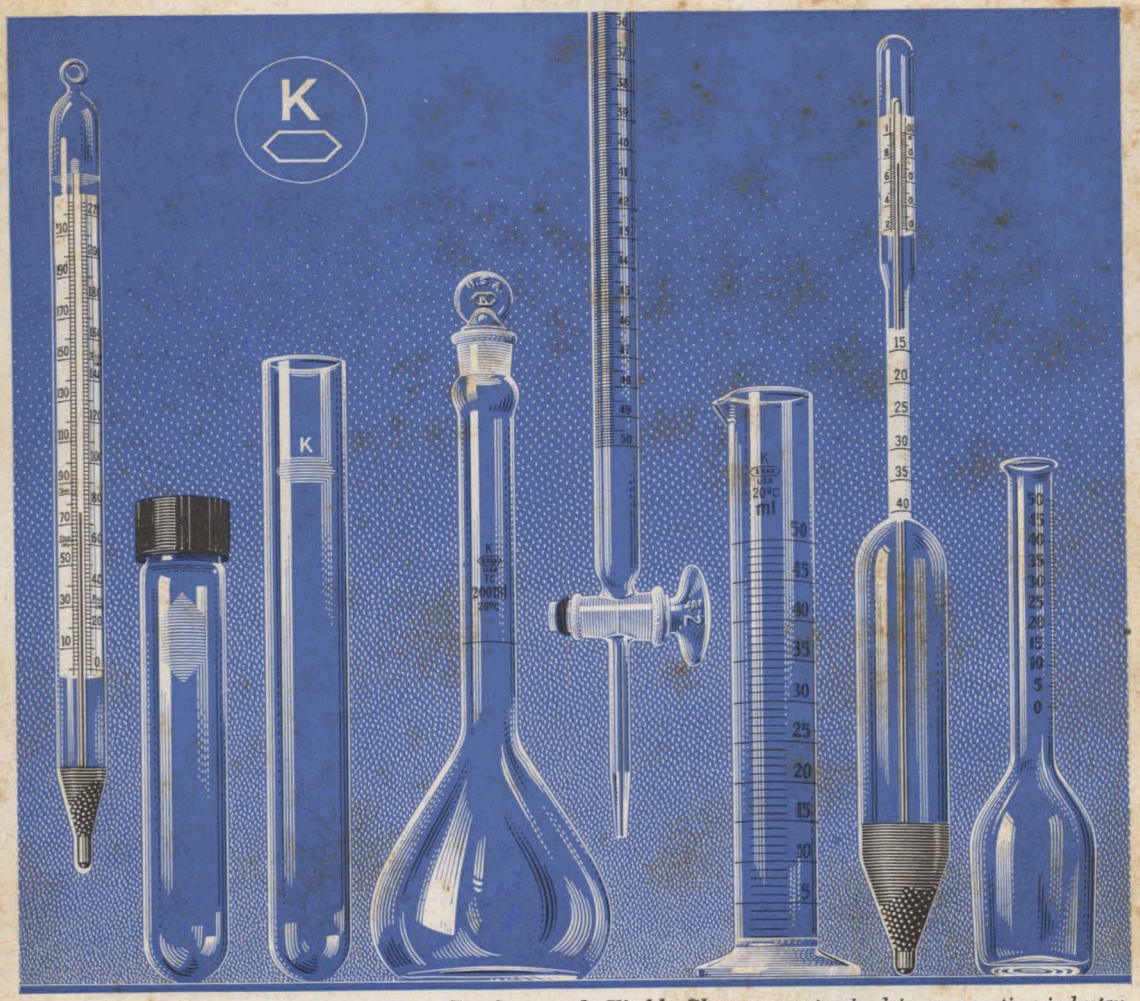

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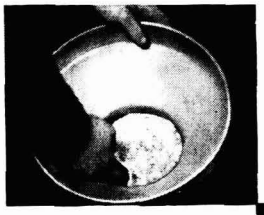

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVII October, 1954 NUMBER 10

REPRODUCTIVE RATES AND GROWTH OF PUREBRED BROWN SWISS CATTLE IN BRAZIL[1]

G. G. CARNEIRO[2] AND J. L. LUSH

Department of Animal Husbandry, Iowa State College, Ames

That cattle of breeds originating in Europe thrive less well in tropical climates than in the regions of their origin is widely believed. The actual evidence is extensive enough to be convincing but is mostly from general observations or from experience of the "trial and error" kind, rather than from experiments designed expressly to test the questions involved. Printed descriptions of such pertinent vital statistics as reproductive and growth rates, mortalities, length of life, etc., for whole populations of cattle in tropical and subtropical regions are rare.

The present article summarizes from a study of Brazilian data on Brown Swiss cattle what seem to be the facts most interesting to North American readers. These cattle are commonly called "Schwyz" or "Raça Suíça Parda" in Brazil. The detailed evidence and topics which will be of interest mainly in Brazil are expected to be published there.

The literature on cattle breeding in the tropics has been reviewed by Howe *(5)* and Rathore *(10)*. In Brazil, several aspects of dairy cattle breeding have been studied by some investigators, among which may be cited Joviano *(7, 8)*, Rhoad *(11)*, Carneiro *(2)*, Villares *(13)*, Jordão and Assis *(6)*, and Carneiro and Lush *(3)*. In general, the mortality of the calves is high and the reproductive rates and the milk production are low. However, the calves produced by crossing European sires on the native stock generally have enough resistance to withstand the environment, and such crossbred cows are higher in milk production than the natives. But, as the amount of exogenous blood increases beyond certain limits, the hardiness decreases and the milk production falls. Those limits and the validity of the general ideas just stated are not well verified. The specific causes of maladaptation are not at all well identified. Hence the feasible remedies for them are even less certain.

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[1] Journal Paper No. J-2477 of the Iowa Agricultural Experiment Station, Ames. Project No. 1053.

[2] Present address: Departmento da produção animal, Belo Horizonte, Minas Gerais, Brazil.

THE MATERIAL STUDIED

The data came from two sources: Volumes 1 to 7 of the herdbooks of the registry society, "Associação do Registro Genealógico Schwyz do Brasil" which was founded in 1938, and from the detailed records kept at five experimental or demonstration farms, maintained by federal or state governments. The southernmost station was Ponta Grossa in Paraná at about 25 degrees south, and the farthest north of the five was Tigipió in Pernambuco, which is not far from eight degrees south. The others were Pedro Leopoldo and Leopoldina in Minas Gerais and Pinheiral in the state of Rio de Janeiro. Nearly all of Brazil lies in the tropical zone but differences in latitude, altitude, rainfall, and soils, make the variation in climatic conditions enormous. Climatic factors may affect the cattle directly by altering physiological processes and bringing discomfort, or indirectly through their effects on the pastures and on the prevalence and development of parasites.

Brazilian cattle are raised almost wholly on grass. Under farm conditions the milk production falls about 20 to 30% during the dry season, as compared with the wet season *(3, 11)*. In the dairy regions, feeding the cattle during the dry season is a general practice. The feeds are silage, grass hay, chopped sugar cane, and small amounts of concentrates. However, the amounts of proteins and minerals used are small.

Efforts to minimize the harmful effects of parasites and diseases are made, but the country is large and lacks transportation facilities, the level of rural education is low, and trained veterinarians are scarce compared with the large number of cattle. Management sometimes includes such poor or questionable practices as milking once a day, failing to test or to keep herd records, using untested sires, failing to cull low producers, and primitive methods of handling milk and milk products.

Most of the agricultural extension work has been done through the cattle shows and by lending or selling purebred or high-grade bulls to the farmers. This is a large part of the work of the government farms. Their experimental work mainly concerns methods of management. The methods of breeding and management used in other countries often do not produce the expected results in Brazil.

The general situation with regard to breeding is that new importations are made frequently. On account of their value for breeding purposes, practically no voluntary culling is done among purebred males or females. Low yields are generally excused on the ground that the cows are not yet adapted to the new environment. However, individuals reacting positively to tests for tuberculosis and for Bang's disease are eliminated. The purebreds are mostly owned by the government farms, which raise females for their own use and males to sell or to lend to farmers for crossbreeding with native cows or with Zebus. Some private farms do have purebred cattle, but the owners expect to get considerable revenue from the sale of these for breeding purposes and do not depend wholly on utilizing them for commercial milk production enterprises.

FINDINGS OF THIS STUDY

Sex ratio. The first seven volumes of the registry books contain 863 purebred Swiss calves born from 1931 to 1947, of which 450 were males and 413 were females.[3] The males were 52.1% of the total. This slight excess agrees with most data on sex ratio in cattle. A homogeneity test indicated that the sex ratio did not fluctuate from year to year any more than would be expected from chance.

Twinning. The twins found among these births were eight cases in which both were males and nine cases in which both were females. Twins of mixed sex could not be counted readily, since registration in the herd book is for each sex separately. Presumably the frequency of twins of all kinds was about twice the frequency of like-sexed twins, or somewhere near 2% of the calvings.

TABLE 1

Distribution of the calvings by months (to the nearest per cent)

Month of the year	Season	Pinheiral	Leopoldina	Pedro Leopoldo	Ponta Grossa	Tigipió[a]
April	Dry	6	12	10	4	9
May	"	27	9	8	3	9
June	"	16	10	10	15	12
July	"	15	9	10	19	11
Aug.	"	11	2	8	18	8
Sept.	"	10	3	6	18	8
Oct.	Rainy	7	12	6	9	6
Nov.	"	4	7	9	9	8
Dec.	"	2	6	9	1	6
Jan.	"	1	11	5	trace	7
Feb.	"	0	12	8	1	7
March	"	trace	9	10	3	9

[a] The dry and rainy seasons in Tigipió are from August to March and from April to July.

Months of birth. The percentage of calves born in each month is shown in Table 1 for each of the five farms. Births during the dry season are generally preferred, since it is supposed to be easier to raise calves at this time of year, provided that feed is sufficient for the calves as well as for their dams. During the dry season, mud and flies are scarce and the temperature is cooler. However, some of the purebred cows are bred whenever they are ready, in order to get the maximum number of calves from them within a given period of years. The data suggest a high degree of success at Pinheiral and at Ponta Grossa in getting the calves born during the dry season, but the calvings at the three other farms occurred in all months of the year in fairly large numbers. The monthly distributions at these five farms differ conspicuously but we do not know whether any of them approached the limit of what the man in charge of the farms could achieve.

Mortality of calves. The data on mortality come from only two farms. The management at these farms differed distinctly in some respects, which seem likely to be important.

[3] Registry rules and conditions were such that we think males and females were equally likely to be registered.

TABLE 2

Mortality up to 24 months of age at the Fazenda Pedro Leopoldo

Sex	Number					Per cent raised
	Total born	Born alive	Abortions and stillbirths	Died before 24 months	Raised	
Males	246	218	28	97	121	49.8
Females	219	200	19	73	127	58.0
Unknown	2	—	2	—	—	—
Total	467	418	49	170	248	53.1

Pedro Leopoldo was kept free from Bang's disease and tuberculosis. Soon after birth the calf was separated from its dam and put in a previously disinfected individual pen. Milk was fed in a clean bucket. A grain ration was supplied and the calves were weaned when 6 to 8 months old. They were sprayed for ticks at regular intervals. Abortions and stillbirths and all deaths up to 24 months were recorded. Table 2 summarizes these data for all calves born from 1931 to 1947. They indicate that around 40 to 50% of the purebred Swiss calves born did not reach breeding age.

The annual mortality at Pedro Leopoldo ranged from as low as 6% to as high as 78% of those born. These year-to-year variations were highly significant statistically, but no regular time trend was evident. Whatever factors increased or decreased the mortality from one year to another seem to have operated irregularly.

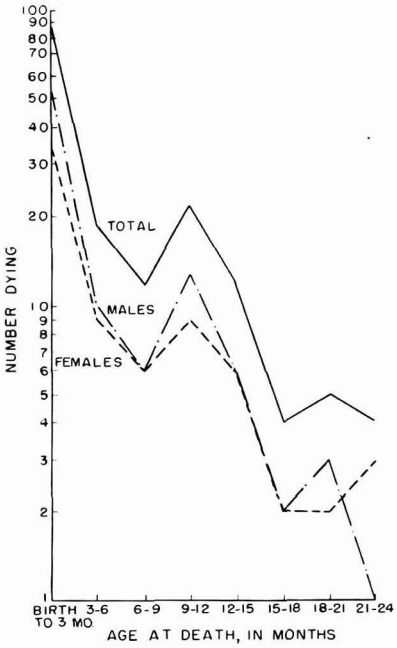

FIG. 1. Mortality at various ages among the calves at Pedro Leopoldo.

The numbers of deaths occurring at each age interval at Pedro Leopoldo are shown in Figure 1 on a semi-logarithmic scale. If the percentage rate of deaths had been constant, these would be straight lines. Steep slopes downward indicate percentage death rates in the interval to which the line leads, lower than in the preceding interval, whereas rising slopes or even slight downward slopes lead to periods when the death rates rose. It appears that the periods of maximum death rates were before 3 months and from 9 to 12 months. The latter is the period when the calves were weaned and starting to live on pastures. It is in these two life stages that investigations of the causes of death seem most likely to be fruitful.

At Pinheiral, the calves were allowed to suckle their dams at will during the day in order to minimize the calf losses. The data at Pinheiral extended only until shortly after weaning and did not include abortions and stillbirths. Eighty-nine deaths in both sexes were reported among 649 live births from 1932 to 1947. This is a mortality of about 14%. If abortions and stillbirths occurred at the same rate as at Pedro Leopoldo, the death percentage at Pinheiral would rise to 24.6%, which is close to the 26.3% found at Pedro Leopoldo for deaths within the first 9 months of age. Thus, as far as they go, the results at Pinheiral confirm those at Pedro Leopoldo in indicating that only about 50 to 60 purebred Swiss calves reach breeding age for each 100 calvings at these farms.

Joviano *(8)* lists, in order of their frequency, the following causes of death from 1937 to 1941 among purebred Swiss of all ages at Pedro Leopoldo: abortions and stillbirths; foot-and-mouth disease; pneumonia; snake poisoning; pneumo-enteritis; weed poisoning; tuberculosis; gastroenteritis (acute and hemorrhagic); congenital debility; and "others." At Pinheiral the inspector in charge reported the most important causes as: tick fever; foot-and-mouth disease; pneumonia; congenital debility; and disturbances in the digestive and urinary tracts.

With a mortality of near 50%, the average number of calvings per cow must be nearly four in order to maintain numbers, even if no voluntary culling is practiced. If mortality rates were lower, there would be more opportunity for intensive selection, there would be less need for continuing large importations of cattle from foreign countries, and the purebred populations could expand more rapidly in numbers. Although veterinarians have done much to minimize the heavy loss of calves, much more needs to be done.

Length of generation. The interval between generations can be measured by the mean age of the parents at the time their offspring were born. Length of

TABLE 3

Mean ages (in months) of sires and dams of male and female calves

	Sires of			Dams of		
	Males	Females	Total	Males	Females	Total
Number	739	706	1,445	739	706	1,445
Average in months	62.5	60.5	61.5	77.6	77.0	77.3
Standard deviation in months	23.0	23.4	23.2	32.6	35.3	34.0

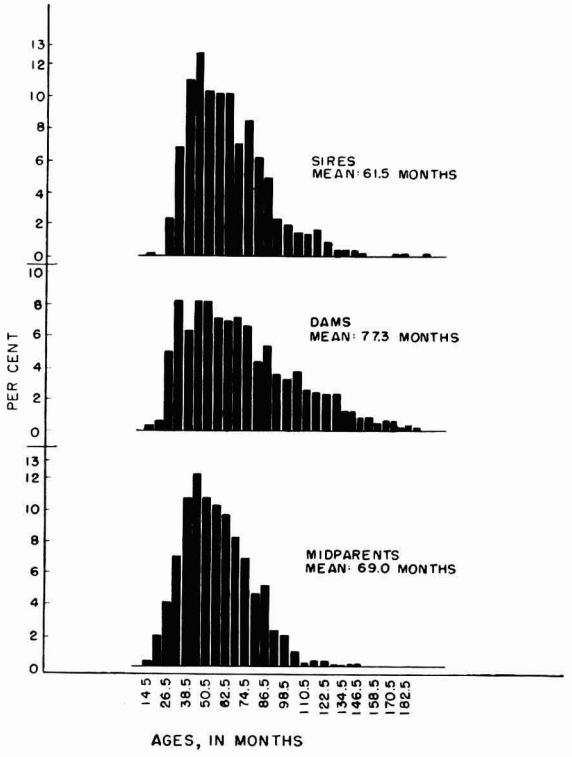

FIG. 2. Distribution of the sires, dams, and midparents according to age.

generation needs to be known if the rate of improvement per generation is to be translated into rate of improvement per year for a breed or any other group of domestic animals. The evidence on this point came from 1,445 purebred Swiss calves, 739 males and 706 females, born from 1937 to 1946 and registered in volumes 1-7 of the Herdbook. The results are shown in Table 3 and Figure 2.

As might have been expected, the ages of the parents of males and of the parents of females did not differ significantly, but the sires averaged much younger than the dams. The mean age of the parents was 69 months, which is a bit higher than the 5.4 years found by Yoder and Lush *(14)* for the Brown Swiss in the United States.

In a newly introduced and expanding breed of cattle, the proportion of older individuals (mostly cows) is expected to be a bit lower than in a population constant in numbers, but to increase with time. The mean age of the parents in these data was calculated for each year separately, and the results show a rather steady increase from 64.5 months in 1937 to 73.8 months in 1946. A straight line fitted by least squares showed an average increase of nearly 0.9 month per year during this 9-year interval, with only a faint hint that this trend in the yearly averages really deviated from a straight line.

Useful life of bulls. The data for this phase of the study came from the Herdbook. Bulls with known age at death numbered 193, and their average productive life after 24 months of age was 75 ± 2.4 months, with a standard deviation of 34 months. This is a longer period than that found by Smith *(12)* in Britain and by Lush and Lacy *(9)* in the United States. This difference might be due in part to the registry system which in Brazil would include many of the bulls which were used only in grade herds at the older ages. Those would not have been detected by the methods used in the studies in Britain or in the United States. Also, purebreds are scarcer in Brazil, so that there is less culling among them. Furthermore, the program of lending bulls to farmers tends to keep as many bulls as possible in active service. If the average productive life actually is 75 months, the annual replacement is about 16% of the bulls in use. Such a long productive life gives opportunity for utilizing progeny testing, since a bull can begin to be proven by his daughters about four years after he is first put into service, provided his first daughters are tested.

Useful life of cows. The average age of 390 cows at first calving on these five farms was 44 months, with a standard deviation of 11.7 months. The averages at the five farms ranged only from 39 to 45 months, and the differences between farms do not seem significant. This average is higher than the figures reported for temperate zones, as was found also by various other students of cattle in the tropics. In Jamaica, Howe *(5)* found a mean age at first calving of 34.7 months for purebred Jerseys and 37.6 months for all Jerseys, including grades and purebreds. He also found 38.6 months for purebred Guernseys, 42.9 months for all Guernseys, and 41.6 months for crossbred Holsteins. He concluded that animals with higher percentages of Zebu blood are slower-maturing sexually than are European cattle. Jordão and Assis *(6)* at São Paulo found that the age of calving was 39 months for imported Holsteins and 35 months for grade Holsteins. Carneiro *(2)* reported an average of 38.7 months for grade Simmenthalers kept under the pen-keeping system. Joviano *(8)* reported for the purebred Swiss heifers at Pedro Leopoldo between 1936 and 1941 a mean age of 3.5 years at first freshening. This is close to the findings of the present study, which included part of Joviano's data.

TABLE 4

Average ages (in months) of cows, by order of calving

Calvings	Mean	n	σ
First	44	390	11.7
Second	62	290	11.8
Third	78	219	13.4
Fourth	93	170	14.3
Fifth	109	126	15.7
Sixth	125	89	17.6
Seventh	140	51	17.5
Eighth	153	28	20.0
Ninth	165	18	14.0
Tenth	179	6	9.6
Eleventh	194	2	19.1

One plausible reason for the high age at first calving is that first service was deliberately delayed in many cases in order to avoid troubles at parturition, which are said to be frequent on account of the size of the Swiss calves. Joviano reports 29 months as the average age at first service for 275 heifers. This, however, is not very different from Engeler's report *(4)* that the mean ages for the Brown Swiss heifers in Switzerland are 28.5 months at the first breeding and 38.2 months at the first calving. The average ages by order of calving are shown in Table 4. Differences between the farms seemed to be only sampling ones; at least there was no hint of statistically significant differences between farms at calvings after the third.

The average productive life of purebred Swiss cows is shown in Table 5, productive life being defined as the period from first calving to the date of cow's disposal or death. Presumably the slightly longer productive life of the imported

TABLE 5
Average productive life of purebred Swiss cows

Groups	No.	Average productive life	Standard deviation
		(months)	*(months)*
All cows	210	57	41
Imported cows	61	62	43
Nonimported cows	149	55	41

cows is due to their having received somewhat better care because more money had been invested in them. Also, the cows in Table 5 are a selected group in that each had calved at least once. Analysis of variance in length of productive life gave no indication that age at first calving had an effect, either among the imported or nonimported cows in Brazil. If 57 months is the average productive life of the purebred Swiss cows, the annual replacement necessary to maintain numbers would be 21.3%. In any extrapolation of this figure to the future, it is to be remembered that the data come from a period in which numbers were expanding and there was little if any voluntary culling.

The average of the 944 known calving intervals on these five farms was 16.8 months. For the imported cows the average was 16.7 months and for those born in Brazil it was 16.9 months, the difference being much too small to be statistically significant. In Switzerland, Engeler *(4)* reported an average interval of 13.8 months for Brown Swiss. The length of calving interval appears to decrease slightly as the cows became older, but this only bordered on statistical significance. Most of this apparent trend came from the unusually long first intervals at Pinheiral. Long intervals between calvings reduce the production of the cow in consequence either of the low amount of milk produced near the end of the lactation or of her having a dry period longer than is optimum. The length of calving interval may indicate something about the reproductive ability of the cows, although genuine differences between the cows themselves may be almost inextricably entangled with differences caused by management practices.

The 210 cows whose lifetimes were complete averaged 3.6 normal calvings during their lives with a standard deviation of 2.5.

Inbreeding. As the total number of Swiss cattle in Brazil is small, it was conceivable that a high level of inbreeding might have been reached, although generally populations must be extremely small and remain completely isolated for many generations for this to occur. The 678 registered females born in the years 1940-1946 were investigated. The average coefficient of inbreeding (Wright's) increased from 0.1% for the 79 head born in 1940 to 2.2% for the 106 head born in 1946. These coefficients are relative to the great-grandparents. Extreme inbreeding is certainly uncommon, but the period of time covered is so short, in generations of cattle, that a high average coefficient of inbreeding for the whole group is scarcely to be expected.

Growth of purebred Swiss calves. Average live weights for the calves at Pedro Leopoldo and Pinheiral at ages up to 12 months are shown in Table 6 for males and females separately. The data include all live weights available at each age.

TABLE 6

Average weights (in kg.) of purebred Swiss calves at 3-month intervals

		Pedro Leopoldo (1931-1947)		Pinheiral (1941-1947)		Total		
Age	Sex	n	Av.	n	Av.	n	Av.	Standard deviation
(mo.)								
Birth	M	233	37.6	143	40.4	366	39	6.4
	F	199	35.1	158	36.2	357	36	5.7
3	M	164	88.9	116	89.7	280	89	23.2
	F	161	85.0	139	84.3	300	85	18.3
6	M	152	151.7	92	139.7	244	147	35.7
	F	149	138.8	106	134.5	255	137	29.8
9	M	144	189.3	104	194.5	248	191	43.8
	F	138	177.3	125	182.1	263	180	34.7
12	M	125	220.9	99	221.4	224	221	45.9
	F	129	201.7	124	206.3	253	204	36.4
15	M	116	251					47
	F	120	228					36
18	M	106	286					50
	F	116	259					37
21	M	93	321					56
	F	112	290					43
24	M	68	361					62
	F	106	315					50
Adult	M	—	—					—
	F	113	438					54

regardless of whether the calf had its weight recorded at all other ages. Weights at ages older than 12 months were available only from Pedro Leopoldo.

The difference between the two farms was small, the calves at Pinheiral being larger at birth, about the same at 3 months, smaller at 6 months, larger at 9 months, and larger, but not significantly so, at 12 months. The two methods of rearing, artificial feeding at Pedro Leopoldo, and being allowed to follow their dams at Pinheiral, seem to produce nearly the same results at 12 months.

The males were consistently and significantly heavier than the females. The difference is of the order of 5 to 8%.

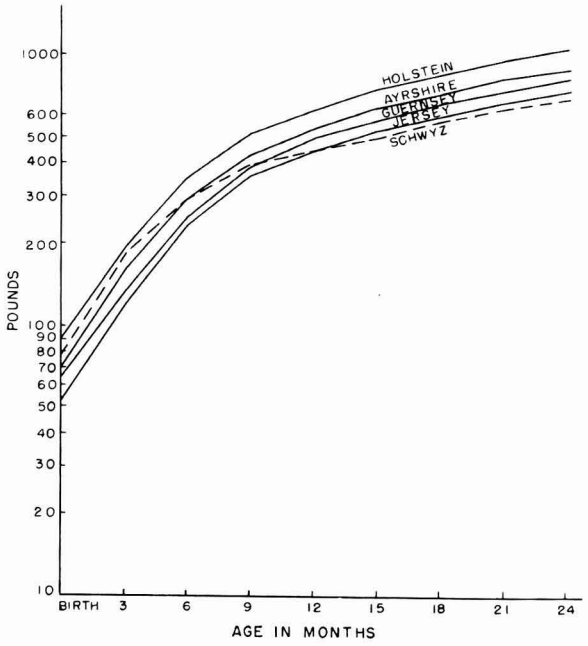

FIG. 3. Growth curves of four dairy breeds in the United States (after Brody) and of the purebred Swiss cattle in Brazil.

Figure 3 shows on a semi-log scale the growth curve of the Swiss heifers compared with the standards of other breeds in the United States as given by Brody *(1,* Table 16.2). As judged by this standard, the Swiss heifers in Brazil grow well during the first 3 months but afterward more slowly than the other breeds reported by Brody, until finally at ages of 15 months and upward they are even lighter than the Jerseys in the United States. Apparently some factors affect adversely the growth of Swiss heifers after they pass 3 months of age and still more extremely at ages of 9 to 15 months. Although these adverse effects

TABLE 7

Analysis of variance in the 12-month weights of heifers

Source of variance	Degrees of freedom	Mean square	Composition of the mean square
Total	242	1,321	$E + \frac{243}{72} S + \frac{243}{18} Y$
Between years	17	4,105	
Within **years**	225	1,111	
Between sires within years	54	1,251	$E + \frac{243}{72} S$
Within sires within years	171	1,009	E

S = 71.7; Y = 211.4

were not identified, the stage at which they occur indicates that they are things which began to happen at or about weaning time, when the heifers were turned into the pastures.

Heritability of differences in live weights. Table 7 shows an analysis of variance of weights at 12 months for all 243 heifers for which individual sires were known. Differences between years were highly significant, but the interpretation of this is uncertain. Many things might have changed from year to year, including such biologically diverse things as changes in management, changes in weather, and resultant feed supply and insect incidence, and changes in the genetic composition of the population.

The intra-year correlation between paternal sisters, $\frac{S}{S+E}$, was 0.066 which, multiplied by four, yields 0.264 as an estimate of the heritability of individual differences between those heifers in their live weights at 12 months. However, this is not statistically significant, as the 95% confidence interval for the paternal sib correlation of 0.066 was from –0.10 to +0.20. On this volume of data this estimate of heritability can be regarded only as a straw in the wind, but no way of exploring heritability further was evident. The large size of the Y component may serve as a warning against using the paternal half-sib correlation without first subtracting or correcting for time trends. The numerical value of the paternal half-sister correlation in these data, if no attention is paid to years, would be: $\frac{S+Y}{S+E+Y}$, which is 0.219. Any use of this correlation, or indeed of the intra-year one, as a basis for estimating heritability, obviously requires some knowledge or working hypothesis concerning how much is genetic in the S and Y components. Multiplication by four to estimate heritability gives tremendous leverage to any errors caused by supposing that all of S or Y are due to genetic differences.

SUMMARY

Pertinent facts about the reproductive and growth rates of purebred Brown Swiss cattle in Brazil are described as a step in locating the influences which lower the adaptability of European breeds of cattle to tropical conditions.

At two of the five publicly-owned farms, about 80% of the calvings occurred during the dry season. At the three other farms, the calvings were distributed nearly equally in the seasons. The sex ratio was 52.1% males among the purebred calves born.

At the one farm which had complete data on calf mortality, 219 of the 467 born were abortions and stillbirths or died before reaching 24 months of age. At another farm, 89 of the 634 born alive had died by weaning time or shortly afterward. These two farms agreed fairly well on the amount of mortality before weaning among those born alive. It appears that only about 50 to 60% of the calvings result in animals which live to at least 2 years of age. A little more than half the total deaths before 24 months occurred between birth and 3 months. The other period when mortality was especially high was from 9 to 12 months,

which coincides roughly with the period when the calves are turned out in the pastures. Year-to-year variation in the death rate was large and statistically significant, but irregular. According to the men in charge at these farms, the most important causes of death were: abortion, stillbirths, tick fever, pneumonia, congenital debility, and foot-and-mouth disease.

The sires averaged 61.5 months and the dams 77.3 months of age when their offspring were born. This amounts to an average interval of 5.75 years between generations. The purebred Brown Swiss cattle population was expanding over this period and the generation interval was increasing at about 0.9 month per year as the population came to include a larger fraction of older breeding animals. If it is assumed that the bulls go into service at 24 months, their average productive life was 75 months and the annual replacement of those in use was about 16%. The average productive life of the cows from first calving to the date of their disposal or death was 62 months for imported cows and 55 months for those born in Brazil. This makes an annual replacement rate among cows of 21%, but this figure makes no allowance for heifers which were intended for breeding but never calved, or for the population being an expanding one.

The heifers averaged 44 months old at first freshening. The average interval between consecutive calvings was 16.8 months. No significant difference in calving interval was found between imported and nonimported cows, and there was no clear evidence of calving interval being affected by the age of the cow.

Little inbreeding has occurred among these Brown Swiss cattle in Brazil.

On the two farms which recorded live weights, the males were significantly heavier, the difference generally being of the order of 5 to 8%. The calves grew well during the first 3 months but after that, and especially after weaning, the growth rates were much lower than those generally regarded as standard in the United States.

Heritability of intra-year differences in live weight among the Brown Swiss heifers at 12 months was estimated at 0.26 on the basis of mean squares within and between sires within years, but this was not statistically significant and can be regarded only as a highly uncertain indication.

REFERENCES

(1) BRODY, S. *Bioenergetics and Growth.* Reinhold Publishing Co., New York. 1945.

(2) CARNEIRO, G. G. Factors Affecting the Milk Production of Simmenthaler Grade Cows under the Penkeeping System. *Iowa State Coll. J. Sci.*, 13: 249. 1939.

(3) CARNEIRO, G. G., AND LUSH, J. L. Variations in Yield of Milk under the Penkeeping System in Brazil. *J. Dairy Sci.*, 31: 203. 1948.

(4) ENGELER, W. Die Ergebnisse statistischer Auswertung 40 jahriger Herdebuchaufzeichnungen beim schweizerischen Braunvieh. *Schweiz. Stammzuchtbuch kommission für Braunvieh.* Luzern. 1933.

(5) HOWE, J. W. The Effects of Varying Amounts of Zebu Blood on the Adaptability of Dairy Cattle to Conditions in Jamaica. *Trop. Agr.*, 26: 33. 1949.

(6) JORDAO, L. P., AND ASSIS, F. P. Contribuição para o estudo do Gado Holandês, Variedade Malhada de Preto e Branco no Brasil. I. Alguns Aspetos da Eficiência Reprodutora das Fêmeas do Plantel da Estação de Experimentação Animal de Pindamonhangaba. *Bol. ind. animal*, 6: 11. 1943.

(7) JOVIANO, R. Fazenda Modelo de Criação de Pedro Leopoldo, 1919-1932. *Serv. Ind. Pastoril. ministerio agr.*, Sec. Agr., Belo Horizonte. 1934.

(8) JOVIANO, R. A Criação da Raca Schwyz em Minas Gerais. *Bol. ministerio agr. (Brazil)*, **32**: 69. 1943.

(9) LUSH, J. L., AND LACY, M. D. The Ages of Breeding Cattle and the Possibilities of Using Proven Sires. Iowa Agr. Expt. Sta., *Bull.* 290. 1932.

(10) RATHORE, A. Differences in Growth of Sindhi and Crosses of Sindhi with Jersey, Brown Swiss, Holstein-Friesian and Guernsey Cattle. Unpublished Ph.D. thesis. Iowa State College Library, Ames. 1949.

(11) RHOAD, A. O. Production of Brazilian Dairy Cattle under the Penkeeping System. *Tierzucht u. Züchstungbiol. (Reihe B, Z. Zücht.)* **33**: 105. 1935.

(12) SMITH, A. D. B. Why So Few Old Cows? Shorthorn Soc. Gr. Brit. and Ire., *Shorthorn Breeders' Guide*, p. 39. 1927.

(13) VILLARES, J. B. Climatologia Zootécnica. II. Os Valores Hemométricos como Índice de Aclimação do Bos Taurus. *Rev. ind. animal, N. serie*, **3**: 7. 1940.

(14) YODER, D. M., AND LUSH, J. L. A Genetic History of the Brown Swiss Cattle in the United States. *J. Heredity*, **28**: 154. 1937.

A STUDY OF 1,000 BOVINE GENITALIA[1]

J. R. PERKINS, DURWARD OLDS, AND D. M. SEATH
Kentucky Agricultural Experiment Station, Lexington

Infertility in dairy cattle is one of the more serious problems facing dairymen today. Trimberger and Davis *(9)* have estimated that about 6% of the cows are sterile and that about 14% of those which do conceive require three or more services. Financial losses occur when valuable animals must be sold as nonbreeders. Fewer calves and less milk are produced when calving intervals exceed 12 months.

The present study was made in order to: *(a)* gain information regarding the variation to be expected in the normal size of reproductive organs in cows; *(b)* confirm and expand information regarding the establishment of fetal membranes; *(c)* correlate the size of the fetus with the diameter of the uterus, as an aid in estimating the stage of gestation by rectal palpation in live cows; and *(d)* tabulate the frequency of gross abnormalities in the female reproductive organs.

EXPERIMENTAL PROCEDURE

The data for this study were gathered from the examination of the reproductive tracts of 1,000 slaughter-house cows and heifers. In routine slaughter of cattle no doubt many cows are included that have been sold as nonbreeders. These data would have been more desirable had they been obtained from a random sample of the cow population. Nevertheless, it is likely that the relative frequency of abnormalities will be similar to that found in an over-all cow population. The comparison of abnormalities found in heifers and in cows should indicate the abnormalities which are associated with pregnancy and parturition.

RESULTS AND DISCUSSION

The various parts of the reproductive tracts of 100 parous cows were measured. The average of these measurements are found in Table 1. Only those free of any visible pathological condition were used. Results from the measurements were in general agreement with those of Sisson and Grossman *(6)*. However, there were some rather outstanding differences. The mean length of the vagina was found to be approximately 7 in., as compared with 10-12 in. as reported by Sisson and Grossman, and the cervix was found to be an inch shorter than the 4 in. which they reported.

The right ovary was slightly larger than the left ovary, and the right uterine horn was greater in length and in diameter than the left horn. It is possible that

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TABLE 1

The average size of the reproductive organs of parous cows

Organ	Number measured	Measurement			
		Mean	Standard deviation	Mean	Standard deviation
		(cm.)	*(cm.)*	*(in.)*	*(in.)*
Vulva					
Length	91	9.65	1.24	3.8	0.49
Vagina					
Length	95	7.58	1.84	6.9	0.72
Cervix					
Length	100	7.99	1.38	3.1	0.54
Diameter	100	3.25	0.83	1.3	0.33
No. of folds	93	3.22	0.70	3.22	0.70
Left uterine horn					
Length	100	39.00	6.82	15.35	2.69
Diameter	100	2.28	0.40	0.89	0.16
Right uterine horn					
Length	100	39.56	7.42	15.57	2.92
Diameter	100	2.40	0.57	0.94	0.22
Left oviduct					
Length	98	20.68	2.30	8.14	0.91
Diameter	98	0.26	0.036	0.10	0.014
Right oviduct					
Length	95	20.71	2.40	8.15	0.94
Diameter	95	0.26	0.036	0.10	0.014
Left ovary					
Length	99	3.44	0.537	1.35	0.21
Width	99	2.25	0.451	0.88	0.18
Thickness	99	1.62	0.385	0.64	0.15
Right ovary					
Length	97	3.60	0.576	1.42	0.23
Width	97	2.40	0.534	0.94	0.21
Thickness	97	1.75	0.388	0.69	0.15

the greater size of the right horn was due to the more frequent occurrence of pregnancy in that horn.

Of the 1,000 specimens studied, 255, or 25.5%, were pregnant. The right horn contained 57.3% of these pregnancies. Other workers *(4, 5)* also have found that the right ovary functions more frequently than the left.

The corpus luteum of pregnancy was found on the ovary opposite the horn carrying the fetus in four of the 255 pregnancies. Presumably, this could be due to intra-uterine migration of ova. Such a phenomenon is of common occurrence in swine *(11)*. Another possibility has been suggested by the work of Clark *(1)*, who found that some pregnant cows may develop a new corpus luteum on the opposite ovary while the original corpus luteum of pregnancy regresses. The physiological mechanism for this occurrence is not understood. It has been reported *(2, 3)* that 3 to 5% of the cows return to heat while pregnant.

A positive correlation was found between the crown-rump length of the fetus (distance from crown of head to rump) and the diameter of the uterine horns.

A calculation of the regression relationship *(7)* showed that there was an increase of 0.374 cm. in the diameter of the pregnant horn for each centimeter increase in crown-rump length of the fetus. There was a corresponding increase of 0.079 cm. in the diameter of the nongravid horn.

For convenience in handling, specimens which were estimated to be more than 4 months pregnant were discarded at the slaughter house. A summary of the measurements on pregnant specimens is given in Table 2.

TABLE 2

Crown-rump length of fetus and diameter of the uterine horns at various stages of pregnancy

Estimated period[a] of pregnancy	Crown-rump length[b]	Pregnant horn diameter	Nonpregnant horn diameter
(Days)	*(cm.)*	*(cm.)*	*(cm.)*
30	1.2	2.79	2.43
42	2.3	3.20	2.52
60	6.6	4.81	2.86
90	16.4	8.47	3.64
120	27.1	12.48	6.58

[a] The estimated ages for fetuses up to 6.0 cm. were taken from Winters *et al. (12)*, and the estimated ages for fetuses over 6.0 cm. were taken from Swett *et al. (8)*.

[b] Straight line measurement from crown of head to rump.

The development and attachment of fetal membranes were studied in 153 single pregnancies. The membranes were found to be in the process of formation at very early stages. They were entering the nongravid horn when the crown-rump measurement of the fetus was 1.2 cm. (about 35 days old). When the fetus was 2.5 cm. long (about 40 days), the membranes began to show evidence of attachment in the pregnant horn, and when the fetus was about 6.0 cm. long (60 days old), the attachment began in the nonpregnant horn.

About 54% of the 556 reproductive tracts from cows and the same per cent from the 444 heifers showed one or more abnormalities of the reproductive tract. Although there was no difference in the percentage of cows and heifers having abnormalities, certain conditions were more prevalent in one than in the other. (See Table 3.)

Based on the presence and severity of abnormalities, it seemed certain that at least 3.3% of the specimens were from cows that were sterile. In addition, about 8.6% of the specimens had abnormalities which, though they might not cause complete sterility, probably would lower fertility. More than half of these abnormalities were infections or injuries which probably could have been prevented by proper management at calving time or possibly could have been successfully treated by competent veterinarians. (These observations are only estimations and should not be considered as established facts.)

Granular vaginitis was the most frequent abnormality encountered in this study. As shown in Table 3, 28.3% of the 1,000 specimens had granular vaginitis, although none of these were listed in the 119 cases of lowered fertility mentioned above. Troutman *(10)* has found from a field study that granular vaginitis may lower the breeding efficiency by 3 to 10 percentage units, depending on its

TABLE 3

Occurrence of abnormalities in 1,000 slaughter house specimens

Abnormality	Frequency of occurrence		
	In all specimens (1,000)	In all cows (556)	In all heifers (444)
	(%)	*(%)*	*(%)*
Granular vaginitis	28.3	23.0	34.9
Cervicitis	8.9	15.45	0.675
Vaginitis	6.7	8.27	4.73
Hymens	6.3	0.00	14.175
Metritis	5.3	9.0	0.675
Ovarian adhesions	4.8	6.77	2.25
Cysts of the vulva	3.4	5.9	0.225
Cystic ovaries	2.2	3.06	1.125
Salpingitis	1.3	1.60	0.9
Vaginal cysts	0.7	1.08	0.225
Incomplete tracts	0.5	0.00	1.125
Double cervix	0.3	0.00	0.675
Adhesion of uterus	0.3	0.18	0.45
Punctured vagina	0.2	0.00	0.45
Cervical cysts	0.2	0.36	0.000
Uterus unicornus	0.2	0.00	0.45
Decomposing fetuses	0.2	0.00	0.45
Mummified fetus	0.1	0.18	0.000
Invaginated uterus	0.1	0.18	0.000

severity. His study was made from artificial breeding results, and the effects might be more severe if natural service were used.

Cervicitis, metritis, and vaginitis were found to occur almost entirely in the reproductive tracts of cows and not in heifers. These findings indicate that these diseases may be closely associated with pregnancy and parturition. The conditions found ranged from mild to quite severe, with some few cases of metritis being severe enough probably to cause permanent sterility.

Granular vaginitis, plus the presence of hymens and anatomical abnormalities, was found to occur more frequently in the organs of heifers than in those of cows. There are no means of treating anatomical abnormalities, and the hymens seemed to be lost at the time of service or parturition.

Cysts other than ovarian were found in 43 of the 1,000 reproductive tracts, 34 of them being of the major vestibular gland. Cysts were found also in the vagina and cervix. This type of cyst has not been reported as having an effect on fertility.

All ovarian cysts, cystic follicles, and cystic corpora lutea were listed together. Of the 22 cases, 14 were follicular and 8 were luteal cysts.

There was a definite decrease in abnormalities of the reproductive tract as examination progressed from the vulva to the oviducts. Thirty-six and nine-tenths per cent of the vulvas, 13.9% of the vaginas, 9.4% of the cervices, 5.9% of the uteri, and 1.3% of the oviducts had one or more abnormality. The ovaries showed abnormalities in 7.0% of the specimens.

The occurrence of abnormalities of the various organs which were definitely severe enough to cause lowered fertility or sterility numbered 119 cases and were

distributed as follows: ovaries, 40%; uterus, 33%; vagina, 18%; cervix, 7%; and oviducts, 2%.

SUMMARY

The average measurement (length, unless otherwise stated) for the various parts of apparently normal reproductive tracts from 100 parous cows were as follows: vulva, 3.8 ± 0.49 in.; vagina, 6.9 ± 0.72 in.; cervix, 3.1 ± 0.54 in. (2 to 4 transverse folds; diam. 1.3 ± 0.3 in.); uterine horns, 15.46 ± 2.80 in. (diam. 0.9 ± 0.2 in.); oviducts, 8.1 ± 0.9 in. (diam. 0.1 ± 0.01 in.); and ovaries, about 1.4 × 0.9 × 0.65 in. The right ovary and right uterine horn were, on the average, slightly larger than their counterparts on the left. Among 255 pregnant specimens studied, 57.3% of the fetuses were found in the right uterine horn. The corpus luteum of pregnancy was found in the ovary opposite the horn carrying the fetus in four cases.

Fetal membranes were studied in 153 specimens having single fetuses of varying sizes. Though there were obvious variations, the membranes appeared to extend into the nongravid uterine horn when the fetus was about 1.2 cm. long (35 days) and showed evidence of attachment in the gravid horn when the fetus was about 2.5 cm. long (40 days).

Among 1,000 slaughter-house specimens, about 54% showed visible evidence of one or more abnormalities. Of the 1,000 organs examined, 3.3% had abnormalities which definitely would have rendered the cow sterile, and another 8.6% had abnormalities severe enough probably to cause lowered fertility.

Granular vaginitis was present in 28.3% of the specimens and was the most frequent abnormality found. Vaginitis (nongranular), cervicitis, and metritis were much more frequent in specimens from cows than in those from heifers. Among specimens from heifers, granular vaginitis, persistent hymens, and other anatomical abnormalities were most often found. In general (all specimens), the incidence of abnormalities decreased as the distance from the exterior increased. (The ovaries were an exception to this rule, however.)

REFERENCES

(1) Clark, C. F. Does the Right Ovary of the Bovine Function More Frequently Than the Left? *J. Am. Vet. Med. Assoc.*, **88**: 62. 1936.

(2) Cunkelman, J. W. The Clinical Diagnosis and Treatment of Breeding Unsoundness in Cows. *J. Am. Vet. Med. Assoc.*, **112**: 292. 1948.

(3) Donald, H. P. Heat During Pregnancy in Dairy Cattle. *Vet. Record*, **55**: 297. 1943.

(4) Henderson, J. A. Observations on Reproduction and Associated Conditions in a Herd of Dairy Cattle. *Cornell Vet.*, **28**: 173. 1938.

(5) Reece, K. P., and Turner, C. W. The Functional Activity of the Right and Left Bovine Ovary. *J. Dairy Sci.*, **21**: 37. 1938.

(6) Sisson, S., and Grossman, J. D. *The Anatomy of the Domestic Animals.* 3rd ed. W. B. Saunders Company, Philadelphia. 1938.

(7) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(8) Swett, W. W., Matthews, C. A., and Fohrman, M. H. Development of the Fetus in the Dairy Cow. USDA *Tech. Bull.* 964. 1948.

(9) TRIMBERGER, G. W., AND DAVIS, H. P. Conception Rate in Dairy Cattle by Artificial Insemination at Various Stages of Estrus. Neb. Agr. Expt. Sta. *Research Bull.* 129. 1943.

(10) TROUTMAN, E. C. Granular Vaginitis as a Cause of Infertility in Dairy Cattle. *J. Am. Vet. Med. Assoc.*, 124: 184. 1954.

(11) WARWICK, B. L. Intra-Uterine Migration of Ova in the Sow. *Anat. Record*, 33: 29. 1926.

(12) WINTERS, L. M., GREEN, W. W., AND COMSTOCK, R. E. Prenatal Development of the Bovine. Minn. Agr. Expt. Sta., *Tech. Bull.* 151. 1942.

THE EFFECT OF STORAGE TEMPERATURES ON THE GROWTH OF PSYCHROPHILIC ORGANISMS IN STERILE AND LABORATORY PASTEURIZED SKIMMILKS[1]

W. C. LAWTON AND F. E. NELSON
Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

Growth of organisms at refrigeration temperature in market milk is becoming more important because of the longer periods of holding before consumption. Rogick and Burgwald *(12)* reported that no psychrophilic bacteria ever were found in 4.1 ml. of pasteurized milk taken from the vat or high-temperature short-time pasteurizer; psychrophilic bacteria were found in some of the bottled milk, particularly in the first milk bottled, indicating post-pasteurization contamination. Erdman and Thornton *(9)* found that only four of 722 psychrophilic isolates were able to withstand pasteurization in the laboratory. These results would seem to account for the findings of Sherman *et al. (13)* that bacterial growth in pasteurized milk was much slower at 0° C. than growth in raw milk at the same temperature and the keeping quality was two to three times as long. Reinoculation of pasteurized milk with minute amounts of raw milk decreased the keeping quality of the pasteurized milk so that it was similar to raw milk.

Dahlberg *(7)* found that coliform bacteria in pasteurized milk stored at refrigeration temperatures increased more rapidly than total count. This may have been due to psychrophilic coliforms or to the fact that they were recontaminants, not subjected to heat treatment, and so were more actively growing than those organisms which survived the heat treatment. In a study of commercial milk supplies, Dahlberg *(6)* also found that coliforms increased on low temperature storage as rapidly as other bacteria. However, standard plate counts were made at 37° C., which would not allow the enumeration of some bacteria growing at lower temperatures.

Burgwald and Josephson *(4)*, Dahlberg *(6)*, and Chaffee *(5)* all reported that the bacterial count did not tend to increase until after 3-4 days. Incubation temperatures used for plates were not reported in all cases, and it is possible that some of the counts are not valid in the light of later knowledge about the growth of low-temperature organisms.

The studies reported in this paper were started when pasteurized milk samples from different commercial sources all were found to contain some organisms that would grow at 3° C. when a 10^6 dilution was plated after the milk had been held for 1 week at 3° C. It was decided that if organisms capable of growth at this low temperature are so prevalent it may be desirable to know more about their activities at different growth temperatures.

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METHODS

Commercial samples of pasteurized milk were obtained, plated on tryptone-glucose-extract (TGE) agar *(8)* and incubated at 3° C. until colonies appeared. Single colonies were picked from these plates and propagated in litmus milk. Each culture obtained was subsequently purified by at least three single-colony isolations. Culture 13, a known *Pseudomonas fragi*, was obtained from the stock collection of the department. All cultures were carried at 5° C. and transferred in litmus milk every 10 days. A 10-day-old culture was used as inoculum for all growth curve studies.

Tests used to characterize organisms were those outlined in the *Manual of Methods for Pure Culture Study of Bacteria (14)*. *Bergey's Manual (3)* was used in classifying the organisms.

Milk in 100-ml. quantities was laboratory pasteurized at 61.7° C. for 30 minutes, rapidly cooled, and held at 3° C. overnight. The following morning the samples were divided into two parts, one part being inoculated with 10^3 cells of one of the isolated cultures, the other part serving as a control. Each part was then further subdivided into screw-cap test tubes in about 5-ml. quantities so that a separate tube was available for each sampling period for both sample and control. Tubes then were stored at 5, 10, 21, 25, and 32° C., a single pair being plated from each temperature for each given time interval. All samples were plated on TGE agar and incubated at 25° C. for 3 days. This time and temperature was used as it had been found previously by Nelson and Baker *(11)* to be most favorable for bacteria which grow at refrigeration temperatures, while also permitting many other organisms to grow. This was supported by Atherton *et al. (1)*. When they plated samples at 32, 26, and 20° C., nearly identical counts were obtained and these counts were equaled by counts at 10° C. after 6 days of holding.

Growth curves also were made with sterile skimmilk prepared by reconstituting dry skimmilk solids to make a 10% solution and autoclaving for 15 minutes at 15 lb. pressure. During these tests 50-ml. quantities of milk were held overnight at the storage temperature to be used, inoculated with about 10^3 cells of a pure culture, subdivided into screw-cap tubes, and incubated at the indicated temperatures. Again, plating was done on TGE agar with incubation at 25° C. for 3 days.

Generation times were calculated for some intervals by the use of the formula:

$$g = \frac{T \log 2}{\log b - \log a}$$

where g is generation time in minutes, T is time interval in minutes, a is count at the beginning, and b is count at the end of time T.

RESULTS

Fifty isolates were made from ten sources of commercial milk. Since screening tests indicated that many of the isolates were very similar, eight organisms were selected as being representative of the various species present.

The organisms used and the number designations were as follows:

Culture 1 was identified as *Pseudomonas ovalis*.

Culture 3 was very similar to *Pseudomonas fluorescens* except that it did not reduce nitrates, and gelatin was completely liquefied in 3 days at 25° C.

Culture 4 was identifiable as *Pseudomonas arvilla*, but its reaction in naphthalene was not checked.

Culture 8 was identifiable as *Pseudomonas cruciviae*, but its action on phenol and *m*-cresol was not checked.

Culture 9 was identifiable as *Flavobacterium aquatile* except that its optimum temperature was not as high as 25° C.

Culture 10 was a *Pseudomonas* species which did not seem to fit in Bergey's classification, as it was not fluorescent, caused rapid and complete proteolysis of milk, liquefied gelatin, and did not reduce nitrates.

Culture 11 was identifiable as *Pseudomonas fluorescens* except that reduction of nitrates continued beyond the nitrite stage. There was a slight suggestion of viscid character on initial isolation, and it is possible that this culture was *Pseudomonas viscosa* that had lost its viscid characteristics.

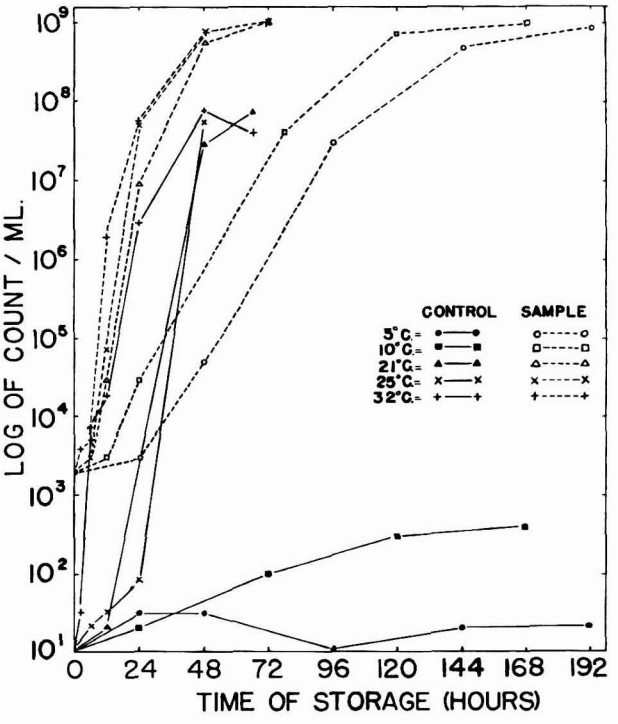

FIG. 1. Culture 3 in laboratory-pasteurized milk.

Culture 12 was similar to *Pseudomonas geniculata*, the only differences being that it did not reduce the litmus milk or cause any visible coagulation except after prolonged incubation at 25° C.

Culture 13 was a typical *Pseudomonas fragi* except that it did not cause an acid coagulation of milk except at 32° C.

Although there was some variation from the characteristics given in Bergey's manual for some of the species, identification as indicated seemed to be warranted.

Figure 1 shows curves obtained at five storage temperatures when culture 3 was grown in laboratory pasturized milk. It was apparent from both sample and control that 21, 25, and 32° C. had a similar effect on the rate of growth in the logarithmic phase. The uninoculated controls and the inoculated samples were separated at these temperatures only by the amount attributable to the inoculum used, as the two groups of curves rose nearly parallel to each other. At 5 and 10° C. the control increased in count very little, whereas the count of inoculated samples rose to a level similar to that reached at the higher temperatures but at a much slower rate. The 5 and 10° C. curves were similar and were separated only by the increased lag phase encountered at the lower temperature. The same or very similar results were obtained with cultures 3, 8, 10, and 11.

As results in Figure 2 demonstrate, when the cultures were added to sterile skimmilk and stored at the various temperatures, the resulting growth curves were strikingly similar to those obtained with laboratory-pasteurized milk.

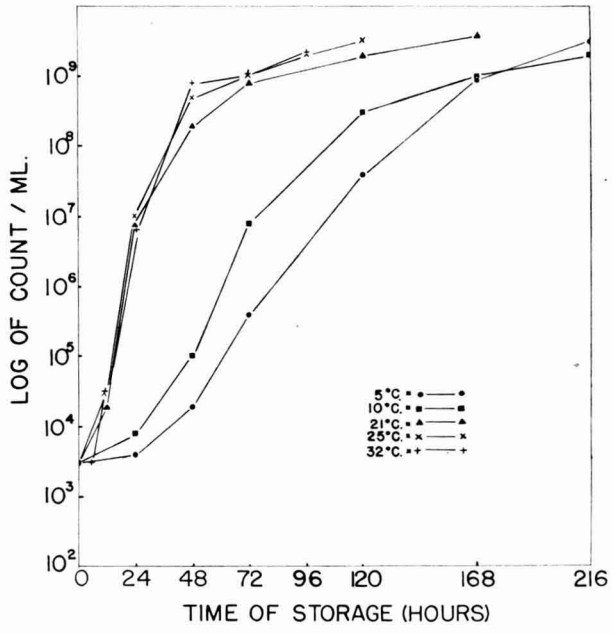

FIG. 2. Culture 3 in sterile milk.

Some of the cultures used did show some variation from the general picture presented in Figures 1 and 2. Culture 9, when grown in sterile skimmilk, grew very slowly with a lag phase of about 48 hours at all temperatures. The rate of growth was much slower, even in the logarithmic phase. Growth did not take place at 32° C., and the rate of growth at 21 and 25° C. was slower than at 5 and 10° C. At the end of 9 days the counts at 21 and 25° C. were only about 10^6, as compared to about 10^9 for 5 and 10° C. storage. Some of the poor results obtained may be explained by the poor growth of this organism on most agar media, making plate counts rather erratic. Cultures 4, 12, and 13 grew slower at 32° C. than at 21 or 25° C. This is illustrated by the data for culture 12 which are graphed in Figure 3. The short logarithmic phase, followed by a

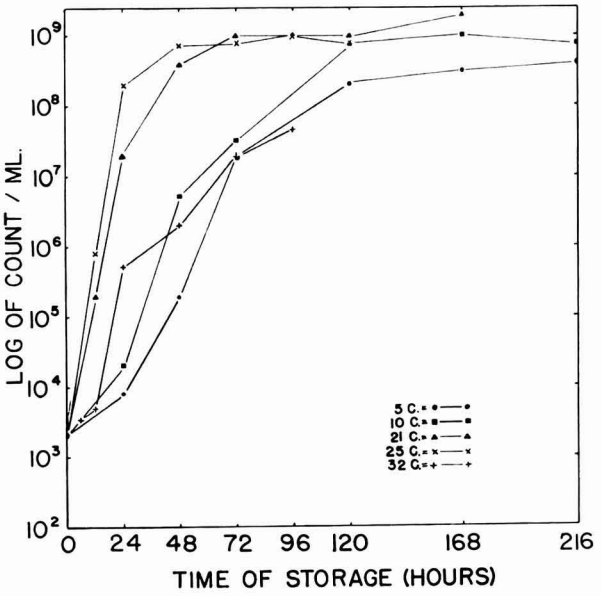

FIG. 3. Culture 12 in sterile milk.

leveling off at 32° C., may have been due to the temperature being very near the upper limit of growth for many low temperature organisms. Erdman and Thornton *(9)* reported that not a single culture of the 722 isolated by them was able to grow at 35.5° C. Rogick and Burgwald *(12)* reported growth at 35° C., but incubation at this temperature tended to change the ability of some of their isolates to produce acid or alkaline reactions, indicating some effect on their growth by this higher temperature. Culture 8 grew rather slowly at 5 and 10° C. but reached a high level after 8-9 days. This organism had a higher optimum temperature than the other isolates, and the lower temperature may be right at the extreme limit of its growth range.

The minimum generation time at each temperature for each culture was

calculated from the growth curves obtained in the sterile skimmilk. The shortest generation time was recorded along with the time period from which it came and reported in Table 1. These figures are to be considered relative, rather than

TABLE 1

Generation time of cultures incubated at different temperatures

Culture No.	Incubation temperature of samples				
	5° C.	10° C.	21° C.	25° C.	32° C.
1	48-72[a] 255[b]	48-72 255	12-24 83	12-24 90	12-24 100
3	48-72 333	48-72 228	12-24 83	12-24 86	12-24 92
4	48-72 231	24-48 217	12-24 96	12-24 108	24-48 188
8	120-168 569	24-48 369	12-24 94	12-24 103	12-24 69
9	168-216 285	72-120 280	48-72 433	48-120 433	 no growth
10	48-72 222	24-48 221	0-24 108	0-12 82	6-12 54
11	48-72 375	48-72 285	12-24 94	12-24 108	12-24 108
12	48-72 217	24-48 175	0-24 108	0-12 83	12-24 104
13	48-72 231	24-48 199	12-24 80	0-12 86	6-12 207

[a] First row of figures for each culture is the interval in which the minimum generation time was obtained.

[b] Minimum generation time in minutes.

absolute. The figures from this table indicated that 21 or 25° C. was the optimum temperature for increase in numbers of the majority of the pure cultures studied and that the logarithmic phase was in the first 24 hours at these temperatures. When the storage temperatures were 5 or 10° C. the logarithmic phase usually was not entered until either the 24-48 or the 48-72 hour interval. Except for cultures 8 and 10, growth at 32° C. was somewhat slower than at 21° C. and usually slower than at 25° C.

DISCUSSION

Common practice in the dairy industry is to cool and hold milk at or below 10° C. in the belief that microorganisms do not grow readily at this temperature. The data presented here demonstrate that low temperatures only retard growth of some organisms. If the milk supply is contaminated with as little as a thousand of these organisms per milliliter they will tend to increase, even at 5° C., to about 10 million after only 3-4 days, a length of time for holding that is not uncommon with our present handling and merchandising procedures. The relative ease with which we were able to isolate large numbers of psychrophilic organisms from all of the 10 samples examined indicated that this type of organism was fairly common in commercial milk supplies, at least in this area.

The lag phases observed are of interest as they are somewhat different than the usual lag phase associated with low temperature organisms. Figure 2 indicates that there is a lag phase of 24 hours when the cultures are held at 5 and 10° C., but little or no lag phase was demonstrable at the higher temperatures. This would seem to indicate that the lag phase associated with low temperature organisms is not a characteristic of the organism but is a physiological condition which can be minimized with an increase in temperature; this can continue to some point between 10 and 21° C. with the cultures used in this study. These short lag phases at 5 and 10° C. and nearly nonexistent lag phases at 21, 25, and 32° C. are in contrast to some of the published work on psychrophilic bacteria. Chaffee *(5)* found that counts on pasteurized milk did not increase for 120 hours and in some cases decreased. Of course this could be due to the fact that there was a very low level of recontamination with psychrophilic organisms or that the temperature of plating, which was not given in the article, may have been unfavorable for the enumeration of psychrophilic bacteria. Burgwald and Josephson *(4)* reported that psychrophilic bacteria did not begin to grow rapidly until after 4-5 days of storage. Dahlberg *(6)* also found a decrease in standard plate count of milk stored at temperatures up to 10° C. and the count did not begin to increase until after 3 days storage. Ayres *et al. (2)*, working with cut-up poultry, found *Pseudomonas* organisms very prevalent and capable of fairly rapid growth at 4.4 and 10° C. These organisms had a lag phase of less than 2 days at both temperatures.

In Figure 1 the inoculated samples growing under identical conditions with the uninoculated controls maintain their initial advantage and in the case of the three higher temperatures grow on nearly parallel courses. However, at 5 and 10° C. the inoculated samples showed a greater rate and a higher level of growth than the uninoculated controls. This type of information clearly shows that low temperature organisms are important because of their ability not only to grow at low temperatures but also to grow rapidly at higher temperatures.

Identification of the typical cultures isolated from plates incubated at 3° C. as predominately members of the genus *Pseudomonas* agrees with the results obtained by Greene *(10)* and Ayres *et al. (2)*. The finding of one culture of *Flavobacterium* also is of interest. The members of these two genera are able to grow at refrigeration temperatures and also are capable of considerable biochemical activity at the low temperatures, some producing pronounced changes in milk. Organisms of these types are common in soil and water and thus may easily gain entrance into dairy products. As these organisms apparently do not survive pasteurization, they probably are post-pasteurization contaminants, being particularly bad where sanitary precautions are not at a high level.

With the exception of the culture of *Flavobacterium aquatile*, these organisms probably all should be classified as "facultative psychrophilic" bacteria. The *Pseudomonas* species all grew more rapidly, in either pasteurized or sterile milk, at temperatures of 21 to 32° C. than at 5 or 10° C. On the basis of final total population, all five growth temperatures gave essentially the same results. The

Flavobacterium culture probably should be classified as truly psychrophilic, for both rate of growth and final population level were greater at 5 and 10° C. than at 21° C. and above.

The lengthening of the lag phase associated with the decrease in incubation temperature for the *Pseudomonas* species is of probable practical importance. Since these bacteria grow well at refrigeration temperatures once they are started, holding at the lowest practical temperature to retard initiation of growth for as long as possible assumes practical importance. Rapid cooling of milk to below 5° C. and holding at or below that temperature will retard growth of low temperature bacteria. When milk is cooled only to 10° C., initiation of growth may be expected in an appreciably shorter period of time, and at 21° C., and above, the lag phase is almost completely eliminated. After growth has been initiated, a return to lower temperatures would only slow down the growth rate of the bacteria which already had left the lag phase. Studies now in progress indicate that the amount of inoculum does not influence the lag phase appreciably. Therefore, a low level of inoculum means that more generations will be required to reach a given population. At 5° C. the generation times commonly are 4 hours or more, and any significant increase in numbers of generations required to reach an undesirable population level increases the life of the product considerably.

CONCLUSIONS

Seven of the cultures isolated from commercial milk and used in this study were members of the genus *Pseudomonas* and one belonged to the genus *Flavobacterium*. All but one of these eight strains were essentially identical with species previously described.

The temperature for optimum growth appears to be about 21-32° C. for most of the organisms isolated. Thus, with the exception of culture 9, the organisms are facultative rather than obligate psychrophiles. Culture 9 probably is an obligate psychrophile, as its optimum growth temperature appears to be around 10° C. and it does not grow at all at 32° C.

The so-called low-temperature organisms can grow rapidly at the higher temperatures and can make significant contributions to the total plate counts if plate counts are made at 32° C. or below.

REFERENCES

(1) Atherton, H. V., Doan, F. J., and Watrous, G. W. Observations on Bacterial Populations and Characteristics of Bottled Milk Under Refrigerated Holding. *J. Dairy Sci.*, 36: 570. 1953.

(2) Ayres, J. C., Ogilvy, W. S., and Stewart, G. F. Post Mortem Changes in Stored Meats. 1. Microorganisms Associated with Development of Slime on Eviscerated, Cut-Up Poultry. *Food Technol.*, 4: 199. 1950.

(3) Breed, R. S., Murray, E. G. D., and Hitchens, A. P. *Bergey's Manual of Determinative Bacteriology*. 6th ed. Williams and Wilkins Co., Baltimore, Md. 1948.

(4) Burgwald, L. H., and Josephson, D. V. The Effect of Refrigerator Storage on the Keeping Quality of Pasteurized Milk. *J. Dairy Sci.*, 30: 371. 1947.

(5) CHAFFEE, C. W. The Bacterial Counts on Pasteurized Milk Held in Refrigerated Storage. *J. Milk and Food Technol.*, **15**: 103. 1952.

(6) DAHLBERG, A. C. The Keeping Quality of Pasteurized Milk in the New York Metropolitan Area During Cool Weather as Determined by Bacterial Counts, Presence of Coliform Bacteria, and Flavor Scores. *J. Dairy Sci.*, **28**: 779. 1945.

(7) DAHLBERG, A. C. The Relationship of the Growth of all Bacteria and Coliform Bacteria in Pasteurized Milk Held at Refrigeration Temperatures. *J. Dairy Sci.*, **29**: 651. 1946.

(8) DIFCO LABORATORIES, INC. *Difco Manual of Dehydrated Culture Media and Reagents.* 9th ed. Difco Laboratories, Inc., Detroit, Mich. 1953.

(9) ERDMAN, I. E., AND THORNTON, H. R. Psychrophilic Bacteria in Edmonton Milk and Cream. *Can. J. Technol.*, **29**: 232. 1951.

(10) GREENE, V. W. The Influence of Various Temperatures on the Growth and Biochemical Activity of Certain Psychrophilic Bacteria Important to the Dairy Industry. Unpublished M.Sc. Thesis. Univ. of Minnesota Library, St. Paul. 1951.

(11) NELSON, F. E., AND BAKER, M. P. The Influence of Time and Temperature of Plate Incubation upon Bacterial Counts of Market Milk and Related Products, Particularly after Holding under Refrigeration. *J. Milk and Food Technol.* (In press.)

(12) ROGICK, F. A., AND BURGWALD, L. H. Some Factors which Contribute to the Psychrophilic Count in Market Milk. *J. Milk and Food Technol.*, **15**: 181. 1952.

(13) SHERMAN, J. M., CAMERON, G. M., AND WHITE, J. C. The Bacteriological Spoilage of Milk Held Near the Freezing Point. *J. Dairy Sci.*, **24**: 526. 1941.

(14) SOCIETY OF AMERICAN BACTERIOLOGISTS. *Manual of Methods for Pure Culture Study of Bacteria.* Biotech. Publications, Geneva, N. Y. 1946.

THE EFFECT OF RELAXIN UPON MILK EJECTION
I. THE LET-DOWN EFFECT UPON SHEEP

D. D. SHAFFHAUSEN, R. M. JORDAN, AND A. E. DRACY
Departments of Animal Husbandry and Dairy Husbandry
South Dakota Agricultural Experiment Station
Brookings

The effects of relaxin upon the symphysis pubis have been demonstrated *(1, 2, 4, 5, 8)* and may be similar to the relaxation of the symphysis pubis occurring naturally at parturition. Frieden and Hisaw *(5)* also noted important actions of relaxin upon connective tissue. In addition, Graham and Dracy *(6)* have shown that relaxin aided in dilating the cow's cervix by causing a relaxation of the cervical muscle.

Assuming that relaxin is present at parturition to cause relaxation of the symphysis pubis, and since Hamolsky *(7)* and Smith *(10)* have shown that relaxin plus estrogen and progesterone increase mammary development more than estrogen and progesterone alone, relaxin may also have a contracting effect on the myoepithelial cells surrounding the alveoli of the mammary gland. Relaxin is known to relax certain smooth muscles; the opposite effect may occur in the mammary gland whereby the milk is forced down, since the teats usually fill prior to parturition. This investigation is concerned with the effect of relaxin upon milk ejection of lactating ewes.

EXPERIMENTAL PROCEDURE

Ten ewes, in various stages of lactation, were milked after the lambs had been removed for 12 hours. Only one half of the udder was milked in order that the lambs might consume the accumulation of the other half after milking. Since the blood supply is common to both halves of the udder, any let-down effect is evident upon either half of the udder. Therefore, only one half of the udder had to be milked to determine the let-down effect of relaxin upon milk ejection.

The ewes were first milked without any induced hormonal effect upon the alveoli, thus allowing each ewe to serve as her own control. Immediately after this milking, 500 G.P.U.[1] of relaxin in a relaxin preparation were injected intrajugularly, and after 1 minute the ewes were again milked to determine the let-down effect of relaxin. In order to evacuate the gland completely, 10 I.U. of oxytocin were given intravenously. After each procedure, the milk obtained was measured to the closest 5 ml.

RESULTS AND DISCUSSION

The amount of milk obtained from each of the treatments for the individual ewes is presented in Table 1. The data show that although there was a wide range in production prior to any hormonal injection, on the average the ewes

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[1] Guinea pig units.

TABLE 1

The effect of 500 G.P.U. of relaxin upon milk ejection in sheep

Sheep No.	Pre-injection[a]	500 G.P.U. relaxin[a]	10 I.U. oxytocin[a]
	(ml.)	*(ml.)*	*(ml.)*
841	80	120	35
823	110	190	85
4952	10	10	5
832	75	145	20
50-7	80	115	25
4923	100	100	30
51-54	35	125	20
1	140	30	145
44-92	40	40	5
52-158	200	90	20
Total	870	975	390
Average	87.0	97.5	39.0
Per cent of total	39.1	43.5	17.4

[a] All milk obtained from one half of the udder after a 12-hour accumulation.

yielded 87.0 ml., or 39.1% of the total production. The injection of 500 G.P.U. of relaxin then initiated further average response of 97.5 ml., or 43.5% of the total milk produced. Oxytocin (10 I.U.) was then injected to evacuate the gland and enable the determination of further let-down of milk, which amounted to 39 ml., or 17.4% of the total production.

The following assumptions are drawn from the data: First, less than half of the milk was obtained from the ewes without the use of an agent to force the milk from the alveoli. This is natural and can be expected from animals not accustomed to being milked, because adrenalin is secreted, which inhibits the action of oxytocin and directly affects milk let-down. The necessity of using oxytocin to evacuate the gland completely is in agreement with data from samples analyzed by Shaffhausen *(9)*.

Second, the effect of this relaxin preparation upon the alveoli appears to be evident by the amount of milk ejected. Since the animals did not completely milk out without some let-down agent, the response to relaxin seems to be self-evident by the amount of milk obtained after the relaxin injection. In every case a response was obtained after the relaxin administration which was nearly equal to or greater than the amount obtained prior to injection. If relaxin did not have a contracting effect upon the alveoli, such a large portion of milk would not, in all probability, have been available. Table 1 shows that although all sheep responded to relaxin, only one (No. 1) gave more milk when oxytocin was injected than when relaxin was injected.

Third, the amount of milk released by the addition of 10 I.U. of oxytocin was enough less than that obtained after the administration of relaxin to suggest that relaxin contracts the alveoli in a manner similar to oxytocin, but not as completely. To date, oxytocin is believed to evacuate the gland by contracting the alveoli *(3)*. Thus, these data suggest that the relaxin preparation may have a contracting effect upon the alveoli as well as a relaxing effect upon the symphysis pubis.

SUMMARY

Ten sheep were hand-milked after the lambs had been removed 12 hours to determine normal milk let-down, effect of 500 G.P.U. of relaxin on let-down, and the use of 10 I.U. of oxytocin to evacuate the gland. On the average, 39.1% of the total milk produced was obtained with no injection; an additional 43.5% of the total milk produced was obtained after 500 G.P.U. of relaxin was injected; and 17.4% of the total milk produced was obtained by evacuating the gland with 10 I.U. of oxytocin.

ACKNOWLEDGMENTS

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REFERENCES

(1) ABRAMOWITZ, A. A., AND HISAW, F. L. Preparations, Biological Assays and Properties of Relaxin. *Endocrinology*, **34**: 103. 1944.

(2) ALBERT, A., MONEY, W. L., AND ZARROW, M. X. An Improved Method of Extraction and Purification of Fresh Whole Ovaries of the Sow. *Endocrinology*, **40**: 370. 1947.

(3) ESPE, E., AND SMITH, V. R. *Secretion of Milk*, 4th ed. Iowa State College Press, Ames. 1952.

(4) FEVOLD, H. L., HISAW, F. L., AND MEYER, R. K. The Relaxative Hormone of the Corpus Luteum, Its Purification and Concentration. *J. Am. Chem. Soc.*, **52**: 3340. 1930.

(5) FRIEDEN, E. H., AND HISAW, F. L. The Mechanism of Symphyseal Relaxation. The Distribution of Reducing Groups. Hexoseamine and Proteins in Symphysis of Normal and Relaxed Guinea Pigs. *Endocrinology*, **48**: 88. 1951.

(6) GRAHAM, E. F., AND DRACY, A. E. The Effect of Relaxin and Mechanical Dilation on the Bovine Cervix. *J. Dairy Sci.*, **36**: 772. 1953.

(7) HAMOLSKY, M., AND SPARROW, R. C. The Influence of Relaxin on Mammary Development in Sexually Immature Female Rats. *Proc. Soc. Exp. Biol. and Med.*, **60**: 8. 1945.

(8) HISAW, F. L. The Corpus Luteum Hormone. *Physiol. Zool.*, **2**: 59. 1929.

(9) SHAFFHAUSEN, D. D. The Quantity and Quality of Ewe's Milk as Affected by Breed, Stage of Lactation, and Somatotrophin. M.S. Thesis. South Dakota State College. 1953.

(10) SMITH, T. C. The Action of Relaxin on Mammary Gland Growth in the Rat. *Endocrinology*, **54**: 59. 1954.

A STUDY OF ACTIVELY CELLULOLYTIC ROD-SHAPED BACTERIA OF THE BOVINE RUMEN

M. P. BRYANT
Dairy Husbandry Research Branch, USDA
Beltsville, Maryland

AND

R. N. DOETSCH
Department of Bacteriology, University of Maryland
College Park

During the study of a variety of bacteria cultured from bovine rumen contents, a number of anaerobic, Gram-negative, nonmotile, actively cellulolytic, rod-shaped bacteria were isolated *(2, 3)*. The importance of these organisms in the ruminal fermentation was suggested by the large numbers in which they were found and because of their rapid degradation of cellulose.

The strains were similar in that they all fermented glucose, cellobiose, and cellulose but not xylose or starch; none of them liquefied gelatin or produced hydrogen sulfide. There was considerable variation in morphology and, more recently, several strains have been found which produce a yellow pigment.

Comparison with cellulolytic bacteria previously described shows these bacteria most similar to *Bacteriodes succinogenes* Hungate *(10)*. On the basis of the few characteristics studied, they differed from the latter in not fermenting starch. Also, since colonies did not develop when strains were inoculated into cellulose agar, comparison could not be made with the unique type of colony formed in cellulose agar by *B. succinogenes*.

The purpose of the present study was to obtain detailed information on the characteristics of this group of organisms.

EXPERIMENTAL PROCEDURE

Eight strains of cellulolytic rod-shaped bacteria were selected for study to include strains showing the range of variation in morphology described previously *(2)*, strains with and without pigment, and strains from different animals maintained on different rations. Strains S23, S61, S85, S111, and S121 were isolated from rumen contents of three heifers fed a ration of alfalfa silage *(4)*; strain C2, from a cow fed wheat straw; and strains M13 and M34, from a heifer fed hay and grain with limited pasture. This animal was located at the University of Maryland, and the others, at the Beltsville station.

The bacteria were isolated from the 10^{-8} dilution of rumen contents and were carried in RGCA slant cultures by using methods previously described *(2)*. Stock cultures were maintained in a dry ice box at –60 to –70° C. Cultures stored in this manner for as long as 13 months remained viable.

To determine fermentation of various carbon sources, the basal medium was

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modified from that formerly used *(2)* in that Trypticase and yeast extract were omitted and 20% of strained rumen fluid was added. Glucose, D-xylose, L-arabinose, maltose, cellobiose, sucrose, trehalose, lactose, galactose, fructose, glycerol, mannitol, and inositol were sterilized by filtration through a Seitz filter and added aseptically to the sterile basal medium. Dextrin, inulin, salicin, esculin, gum arabic, xylan, and pectin were sterilized in the autoclave before addition to the sterile medium. Pectin was added to give a final concentration of 0.75% in the medium; and the other substrates, 0.5%. Fermentation was detected by observing for growth and lowering of pH. Brom thymol blue was added to the test media after 1 week of incubation at 37° C. Soluble starch was added to the medium in 0.1% concentration before sterilization and its hydrolysis was detected by the addition of iodine solution to the medium after 1 week of incubation. Cellulose digestion was detected as previously described *(2)* except that 20%, instead of 40%, of rumen fluid was used in the medium. Controls included uninoculated media with each carbon source and inoculated medium without carbon source.

Tests for gelatin liquefaction were determined after 1 and 2 weeks of incubation in the basal medium with 1.0% of Trypticase, 0.1% of glucose, and 5.0% of gelatin added.

Casein digestion was determined in the basal medium with 0.2% of casein and 0.1% of glucose added. After 2 weeks of incubation, 1.0 ml. of 5 *N* sulfuric acid was added to tubes containing 6 ml. of medium and the amount of casein precipitated was compared with the amount in uninoculated control medium.

Tests for indol production and nitrate reduction were made after 1, 3, and 7 days of incubation in the basal medium with 2% of Trypticase, 0.1% of glucose, and 0.1% of potassium nitrate added. Tests for nitrite and residual nitrite were made.

Cultures for determination of fermentation products were prepared in all-glass culture vessels with inlet and outlet tubes. The medium contained 20% rumen fluid, 0.05% cysteine hydrochloride, minerals *(2)*, 0.4% cellulose (Whatman No. 1 filter paper), and 0.2% sodium bicarbonate and was prepared under nitrogen gas. The medium, minus sodium bicarbonate and cysteine, was adjusted to pH 6.2 and autoclaved, and, after cooling, sterile solutions of sodium bicarbonate and cysteine hydrochloride were added. The medium was inoculated with a 24-hour cellulose medium culture and the vessels were evacuated until the medium boiled so that gases were swept out. The vessels were then sealed with a flame. Control cultures were identical with experimental cultures except that the controls were stored in the refrigerator and the experimental cultures were incubated at 37° C. for 2 weeks.

The amount of cellulose fermented was estimated by the dry-weight difference between the centrifuged residue of the experimental and control media. Gaseous products were estimated as formerly described *(1)*. Benedict's qualitative reagent was used to test for reducing sugars. The methods of Friedemann *(7)* were used to estimate alcohols, total volatile acids, and formic acid. Nonvolatile acid was extracted from the medium with diethyl ether. Lactic acid

was determined by the method of Friedemann and Graeser *(8)* and succinic acid by precipitation of the barium salt from solution by adding five volumes of 95% ethanol and weighing the dry product.

A second determination of acid end products was carried out on strain S85 grown in the medium containing the carbon dioxide gaseous phase and 0.4% sodium carbonate in which larger amounts of cellulose were fermented. The acids were determined chromatographically by the method of Bulen *et al. (5)* modified to contain alphamine red R indicator in the column *(13)*. The acids were eluted from the column by successive additions of 6 ml. chloroform, 35 ml. of 1.0% *n*-butanol in chloroform (CB_1), 20 ml. of CB_{10}, and 80 ml. of CB_{15}.

Inocula used in studying growth requirements consisted of one standard loopful of a 24-hour glucose medium culture when glucose was the carbon source and 0.1 ml. of a 24-hour cellulose medium culture when cellulose was the substrate. The amount of growth was estimated by the amount of acid produced. Ten ml. of culture was acidified with 10 ml. of 0.2 *N* sulfuric acid, brought briefly to a boil to drive off carbon dioxide, and titrated to the phenolphthalein end point with 0.1 *N* sodium hydroxide. The acidities produced were calculated as the difference between the average of duplicate tubes of the inoculated and uninoculated media. Although quantitative comparisons were not made, the amount of acid produced was roughly proportional to the amount of visible growth.

Extracts from alfalfa leaf meal and fresh bovine feces used in the study of growth requirements were prepared by steaming 10% suspensions in distilled water in an Arnold sterilizer for 30 minutes and filtering through Whatman No. 12 filter paper.

RESULTS

Morphologically, the strains were difficult to describe because of changes that occurred on aging and the variation in shape of cells in young cultures. Cultures observed from the water of syneresis of 18- to 24-hour RGCA slant cultures were Gram-negative, aniodophile, nonmotile rods with more or less pointed ends. They usually occurred singly or in pairs, but one strain (M13) showed some chains of three to five short rods. Strains S61 and S111 were smaller, slender rods 0.5-0.6μ long and a few "rosette" arrangements of cells were found occasionally in these cultures. Strain S85 was a short thick rod (1-1.2μ × 1-2μ) with many coccoid cells and a few longer rods. Strains M13, M34, S23, S121, and C2 were slightly smaller in width but tended to be longer than strain S85. As the cultures aged, cells became quite variable with many swollen forms and round bodies that stained very lightly.

Growth in glucose medium was always evenly turbid in 24 hours. Deep colonies in RGCA agar were lenticular and surface colonies were entire, slightly convex, and translucent to opaque, and often showed a "frosted glass" appearance when observed by transmitted light. They were usually about 3 mm. in diameter after 3 days of incubation. Three of the strains, S23, S61, and M34, produced a light yellow pigment in both liquid and solid media.

No visible growth or change in pH occurred in the basal medium without fermentable carbon source. Only glucose, cellobiose, cellulose, and pectin were fermented by all eight strains. Strain S111 fermented maltose, and strains S85, S121, and M13 produced a delayed and weak fermentation of lactose. With the methods used, gelatin was not liquefied but casein was attacked by all strains. None of the strains reduced nitrate or produced indol or acetylmethylcarbinol.

TABLE 1

Products of the cellulose fermentation by two strains of actively cellulolytic rods[a]

	Experiment 1		Experiment 2
Cellulose utilized (as $C_6H_{10}O_5$)	Strain S111 1.72	Strain S85 1.78	Strain S85 2.73
Fermentation products			
Carbon dioxide	−0.69	−0.83	—
Combustible gas	0	0	—
Alcohols	0	0	—
Formic acid	0.23	0.21	0.28
Acetic acid	0.86[b]	0.61[b]	1.00
Propionic acid			0.01
Butyric acid			−0.03
Lactic acid	0	0	0
Succinic acid	0.89	1.26	2.46
Reducing sugar	0	0	0

[a] Expressed as millimols per 150 ml. of medium.
[b] Total volatile acid minus formic acid.

The products of the cellulose fermentation produced by the two strains showing the widest difference in morphology are shown in Table 1. It is evident that the strains produced similar fermentations with the production of large amounts of succinic acid and volatile acid and showed an uptake of carbon dioxide. In Experiment 2, the volatile acid produced by strain 85 was shown to be composed predominantly of acetic and formic acid. The small gain in propionic acid and loss of butyric acid probably were due to error in analysis. The succinic acid isolated from the cultures sublimed and melted at the same temperatures as known succinic acid.

Some growth requirements were studied with one of the faster growing strains (S85). The organism grew well in glucose medium at 30-38° C. but did not grow at 22 or 45° C. It grew in glucose medium with an initial pH of 6.05 to 7.7 but not at pH 5.5. In cellulose media the lower pH limit was about the same. However, growth occurred at pH 7.0 but not at pH 7.5. The final pH after growth in poorly buffered glucose broth was 5.5.

Bicarbonate was a necessary ingredient of media for growth of the organism. When the amount of sodium bicarbonate in glucose medium, used under nitrogen gas instead of carbon dioxide, was varied from 0 to 0.2%, acid produced varied from 0 to 0.36 meq. per 10 ml. of medium.

None of the strains would grow in glucose medium in which 0.5% of yeast extract and 1.5% of Trypticase replaced rumen fluid. The lowest concentration of rumen fluid in the glucose or cellulose medium that allowed maximum growth was about 20%, and 5% allowed about half maximal growth.

TABLE 2

The effect of addition of various substances to 5% rumen fluid–glucose medium on the growth of a cellulolytic rod (strain S85) as measured by acid production[a]

Ingredient	Concentration	Acid produced per 10 ml. of medium
	(%)	*(meq.)*
Control		0.42
Rumen fluid	5	0.71
Rumen fluid	15	0.86
Rumen fluid	35	0.87
Ash from rumen fluid	0.4	0.31
Complex mineral solution[b]		0.38
Bovine saliva	5	0.56
Bovine saliva	20	0.74
Bovine feces extract	10	0.35
Bovine feces extract	50	0.39
Alfalfa meal extract	10	0.29
Alfalfa meal extract	50	0.29
Bovine serum	10	0.00
Yeast extract	0.2	0.22
Yeast-extract	0.5	0.16
Beef extract	0.1	0.40
Beef extract	0.5	0.00
Trypticase	0.5	0.50
Phytone	0.5	0.22
Peptone	0.1	0.26
Peptone	0.5	0.26
Gelatin	0.5	0.42
Casein	0.1	0.50
Casein	0.5	0.94

[a] Acid production determined after 48 hours of incubation.

[b] KH_2PO_4, 0.05%; $(NH_4)_2SO_4$, 0.1%; $MgSO_4$, 0.02%; $CaCl_2$, 0.002%; $FeSO_4 \cdot 7H_2O$, 0.004%; and $MnSO_4$, $NaMoO_4 \cdot 2H_2O$, $CoCl_2$, $ZnSO_4$, and $CuSO_4 \cdot 5H_2O$, 0.0002%.

The growth of the organism in 5% rumen fluid–glucose medium with various substances added is shown in Table 2. Only casein, bovine saliva, and Trypticase stimulated growth. However, another sample of saliva failed to allow a stimulation of growth, and neither saliva, casein nor Trypticase allowed growth in the absence of rumen fluid. When "vitamin-free" casein was added, no stimulation of growth occurred.

Many of the substances caused more or less inhibition of growth, and it was possible that they contained the factors necessary for growth but the inhibition did not allow their detection. McNeill *et al. (12)* observed a similar inhibition of growth of rumen bacteria. Because of this possibility a medium containing known growth factors was tested. It contained the usual minerals, cellulose, resazurin, and sodium carbonate plus the following materials: ferrous sulfate, cupric sulfate, zinc sulfate, cobalt chloride, sodium acetate, guanine, uracil, thymine, xanthine, adenine, *p*-aminobenzoic acid, folic acid, biotin, pantothenate, pyridoxal, pyridoxamine, riboflavin, thiamin, nicotinamide, vitamin B_{12}, inositol, choline, and Trypticase. Other media tested contained the above ingredients plus glutamine glutathione, adenylic acid (yeast), coenzyme I, Tween 80, and oleic acid. These media did not allow growth of the organism, and no appreciable stimulation or inhibition of growth occurred when 5% of rumen fluid was added as compared with a 5% rumen fluid medium control.

The factor(s) present in rumen fluid necessary for growth of the organisms is quite stable, as no appreciable diminution of growth occurred when media contained rumen fluid that was autoclaved at a pH of 3 or 9 for 1 hour at 15 lb. pressure or rumen fluid dried on a steam bath and reconstituted with distilled water.

DISCUSSION

These studies indicate that, although variation in morphology and pigment production occurs within this group of organisms, they are very similar in physiological and cultural characteristics and should be considered as one species.

Their production of large amounts of succinic and acetic acid and uptake of carbon dioxide in the cellulose fermentation, their morphology, temperature range, and inability to grow in media devoid of rumen fluid indicate their close relationship to *Bacteriodes succinogenes* Hungate *(10)*.

They differ from the culture studied by Hungate in that this organism fermented glucose, cellobiose, maltose, cellulose, trehalose, dextrin, and starch, whereas none of the present strains fermented the last three carbohydrates. The present strains produced a measurable amount of formic acid and Hungate's organism may have produced a trace. These apparent differences are minor when compared with similarities and it is concluded that the present strains should be identified as *B. succinogenes*.

The importance of this bacterium in the rumen fermentation is evident from a consideration of the numbers in which they have been isolated, their rapid fermentation of cellulose and pectin, and their production of fermentation products found in the rumen or further metabolized therein. Previous studies *(3, 4)* have shown them to be among the predominant bacteria cultured from the rumen of cows fed alfalfa hay, alfalfa hay and grain, alfalfa silage, and wheat straw; more recently, they were found among the predominant bacteria in cows fed fresh alfalfa or blue grass pasture plus grain. They have been isolated from cows in Texas and Washington State *(9, 10)* and in the present studies in Maryland

Succinic acid has been shown repeatedly to be catabolized by rumen organisms to form propionic acid and carbon dioxide *(6, 11, 15)*, both of which are normal end products of the rumen fermentation, and formic acid was shown to be rapidly dissimilated by rumen bacteria *(6)*.

Several studies have suggested that unknown growth factors are required by ruminal bacteria. In a study of the nutritional requirements of these bacteria as assayed by total colony counts obtained from rumen fluid, McNeill *et al. (12)* found that rumen fluid contained essential factors not found to any degree in rich nitrogenous materials ordinarily used for the growth of nutritionally fastidious bacteria. Using the artificial rumen technique, Ruff *et al. (14)* presented evidence suggesting that an unidentified factor is present in certain feed stuffs that is stimulatory to cellulose digestion. The present study substantiates the observation of Hungate *(10)* that *B. succinogenes* will not grow when various substances are substituted for rumen fluid in the growth medium and suggests that an unknown growth factor is involved. This factor is acid, alkali, and heat

stable. Studies suggested that it is not a common B vitamin, amino acid, peptide, purine, pyrimidine, or mineral, although some of these materials also may be required in addition to the unknown factor.

Work is being continued to determine the nature of this unknown factor and other growth requirements of these important cellulolytic bacteria of the rumen. This knowledge should help to bring about a better understanding of the factors affecting cellulose digestion in the rumen.

SUMMARY

Eight strains of anaerobic, Gram-negative, nonmotile, actively cellulolytic, rod-shaped bacteria found in large numbers in rumen contents were selected for study on the basis of variation in morphology, pigment production, and isolation from different animals fed different rations.

Of many carbon sources tested, only glucose, cellobiose, cellulose, and pectin were fermented by all strains. Large amounts of succinic and acetic acid and smaller amounts of formic acid were produced, and carbon dioxide was taken up, in the fermentation of cellulose. It was concluded that all strains belonged to one species, *Bacteriodes succinogenes* Hungate.

Studies on the growth requirements showed that bicarbonate is required and suggested that rumen fluid contains an unknown heat, acid, and alkali-stable factor that is not a common B vitamin, amino acid, peptide, purine, pyrimidine, or mineral and was not detected in several materials commonly used to grow nutritionally fastidious bacteria or in extracts from alfalfa meal or bovine feces.

REFERENCES

(1) Bryant, M. P. The Isolation and Characteristics of a Spirochete from the Bovine Rumen. *J. Bacteriol.*, **64**: 325. 1952.

(2) Bryant, M. P., and Burkey, L. A. Cultural Methods and Some Characteristics of Some of the More Numerous Groups of Bacteria in the Bovine Rumen. *J. Dairy Sci.*, **36**: 205. 1953.

(3) Bryant, M. P., and Burkey, L. A. Numbers and Some Predominant Groups of Bacteria in the Rumen of Cows Fed Different Rations. *J. Dairy Sci.*, **36**: 218. 1953.

(4) Bryant, M. P., and Burkey, L. A. The Bacterial Flora in the Rumen of Heifers Fed a Ration of Alfalfa Silage. *BDI-Inf* 151. 1953.

(5) Bulen, W. A., Varner, J. E., and Burrell, R. C. Separation of Organic Acids from Plant Tissues, Chromatographic Technique. *Anal. Chem.*, **24**: 187. 1952.

(6) Doetsch, R. N., Robinson, R. Q., Brown, R. E., and Shaw, J. C. Catabolic Reactions of Mixed Suspensions of Bovine Rumen Bacteria. *J. Dairy Sci.*, **36**: 825. 1953.

(7) Friedemann, T. E. The Identification and Quantitative Determination of Volatile Acids and Alcohols. *J. Biol. Chem.*, **123**: 161. 1938.

(8) Friedemann, T. E., and Graeser, J. B. Determination of Lactic Acid. *J. Biol. Chem.*, **100**: 291. 1933.

(9) Hungate, R. E. Studies on Cellulose Fermentation. III. The Culture and Isolation of Cellulose-Decomposing Bacteria from the Rumen of Cattle. *J. Bacteriol.*, **53**: 631. 1947.

(10) Hungate, R. E. The Anaerobic Mesophilic Cellulolytic Bacteria. *Bacteriol. Revs.*, **14**: 1. 1950.

(11) JOHNS, A. T. Isolation of a Bacterium Producing Propionic Acid from the Rumen of Sheep. *J. Gen. Microbiol.*, **5**: 317. 1951.

(12) MCNEILL, J. J., DOETSCH, R. N., AND SHAW, J. C. Some Nutritional Requirements of Bovine Rumen Bacteria. *J. Dairy Sci.*, **37**: 81. 1954.

(13) RAMSEY, L. L., AND PATTERSON, W. I. Separation and Identification of the Volatile Saturated Fatty Acids (C_1 to C_4). *J. Assoc. Offic. Agr. Chemists*, **28**: 644. 1945.

(14) RUFF, E. W., HALE, W. H., AND BURROUGHS, W. Observations upon an Unidentified Factor in Feedstuffs Stimulatory to Cellulose Digestion in the Rumen and Improved Liveweight Gains in Lambs. *J. Animal Sci.*, **12**: 731. 1953.

(15) SIJPESTEIJN, A. K., AND ELSDEN, S. R. The Metabolism of Succinic Acid in the Rumen of Sheep. *Biochem. J.*, **52**: 41. 1952.

ASSAY OF VARIOUS MOLD-RIPENED CHEESES FOR ANTIBIOTIC ACTIVITY[1]

H. H. WILKOWSKE AND W. A. KRIENKE
Department of Dairy Science
Florida Agricultural Experiment Station
Gainesville

In recent years there has developed an interest in the possible effects on humans of intakes of very small amounts of antibiotics in foods over a period of time. This interest caused the American Dairy Science Association through its Manufacturing and Production sections to create a joint Committee on Antibiotics in Milk, charged with the responsibility of obtaining factual information on every aspect of the problem dealing with antibiotics in milk. The Committee's reports of 1951 *(5)* and 1953 *(6)* contained sections dealing with the human sensitization aspect. In the latter report it was pointed out that the Food and Drug Administration *(7)* had issued a statement of policy in which it was pointed out that "the presence of antibiotic drugs in foods intended for human consumption, or the direct or indirect addition of such drugs to such food, may be deemed adulteration." The reasons expressed were that consumption of foods containing antibiotic drugs may cause sensitization of the consumer to such antibiotics and also may result in the emergence of strains of pathogenic microorganisms resistant to these drugs.

As some cheeses are of the mold-ripened type and there are numerous species of *Penicillia* and *Aspergilli* capable of producing penicillin or penicillin-like substances *(12)*, some unfounded suggestions of possible relationships may cause consumer discrimination against these excellent foods unless data are available to eliminate doubt and suspicion.

The research on antibiotics in milk has been reported in recent review articles by Calbert *(3)*, by Claybaugh and Nelson *(4)*, and by Trout *(13)*.

Attempts to increase the yield of penicillin by various culturing practices were reported by Baron *(2)* to have been successful to the extent of the production of 1,000 units per milliliter by the submerged mold growth process as compared to 200 units per milliliter when using the surface mold growth method. Other attempts included new strain developments by ultraviolet irradiation of spores. As a result of this application, Backus *et al. (1)* reported the emergence of a new strain from *Penicillium chrysogenum*, which passed its parent in penicillin production.

By applying ultraviolet irradiation to the spores of green colored *Penicillium roqueforti*, Knight *et al. (9)* obtained white colored mutants of the mold which Morris *et al. (11)* have used successfully in the manufacture of a new type mold-ripened cheese, which has been named "Nuworld" cheese.

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[1] Florida Agricultural Experiment Station. Journal Series, No. 268.

It was the purpose of this investigation to assay various mold-ripened cheeses with respect to substances active against the lactic acid streptococci of dairy starters, which are suitable as test organisms, as suggested by the work of Katznelson and Hood *(8)*.

EXPERIMENTAL PROCEDURE

The 14 samples of cheeses used in this study were commercially produced and were being offered for sale to consumers. They included five different brands of Blue cheese, four manufactured in the United States and one imported from Denmark; three brands of Roquefort imported from France; three brands of United States manufactured Camembert; and two brands (two different samples of one brand and one of the other) of Nuworld cheese. Each sample was satisfactory with respect to mold development and flavor. Trials were made using several samples of each brand of cheese.

With the aid of a Waring blendor, a 20-g. sample of cheese was mixed with 40 ml. distilled water. Measurement of the pH of this mixture was made by the glass electrode method. As the Blue and Roquefort cheeses were of low pH, sufficient $N/10$ sodium hydroxide was added to adjust the pH to within the range 6.5 to 7.5. No sodium hydroxide was added to the Camembert samples. In each case the amount of alkali added was such that the subsequent initial titratable acidity of the sample after dilution with homogenized milk was within the range of 0.15 to 0.18% expressed as lactic acid, which in some instances required as much as 40 ml. of $N/10$ sodium hydroxide.

Some trials included mold-ripened cheese preparations to which penicillin[2] had been added. The penicillin was dissolved in sterile distilled water immediately prior to use. Appropriate dilutions were made so that a final penicillin concentration of one unit per 10 g. of cheese was obtained.

The neutralized cheese and water mixtures (with and without added penicillin) were heated in flowing steam for 5 minutes and filtered through a cotton milk strainer to remove coarse particles of mycelia and curd. The filtrate was cooled and made up to 200 ml. with homogenized milk. Other desired dilutions were made by using this 10% cheese preparation. These received further heat treatment for 75 minutes in flowing steam. After cooling to the incubation temperature of 30° C., the samples were inoculated with 3% active dairy culture and dispensed into test tubes in 9-ml. quantities. At appropriate intervals sets of tubes were removed and the contents of the tubes, together with 9 ml. distilled water rinses, were titrated with $N/10$ sodium hydroxide to exactly pH 8.3 endpoint, determined electrometrically.

RESULTS AND DISCUSSION

Typical of the results obtained on all the samples of cheese are those shown in Figure 1. As lactic acid developed, there were no appreciable differences in

[2] Lederle "Buffered Crystalline Penicillin G Potassium."

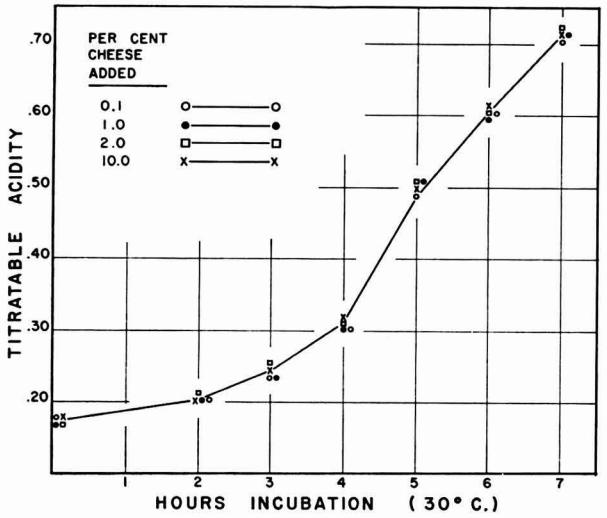

FIG. 1. Rate of lactic acid production by dairy cultures containing various concentrations of Roquefort cheese.

amount produced due to variations in concentration of the cheese in the preparations. It is recognized that 10% cheese would normally not be added to starter, but this procedure was used for the purpose of assaying for antibiotic activity. In other trials extending for a longer period of time than the one shown, the titratable acidity values tended to reach a maximum at about 0.85% in a manner characteristic of an active cheese or buttermilk starter.

When penicillin was added to the cheese preparations in several different concentrations as shown in Figure 2, lactic acid development was practically nil

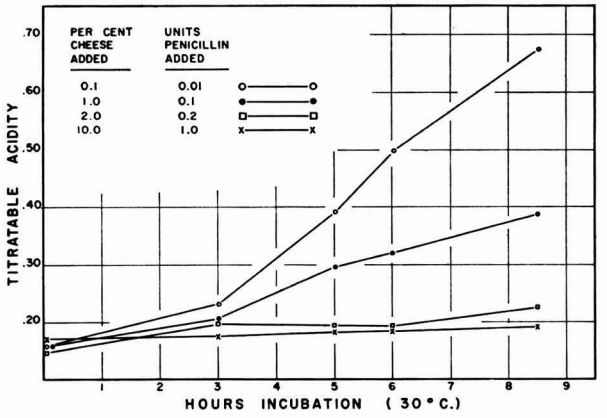

FIG. 2. Rate of lactic acid production by dairy cultures containing various concentrations of Roquefort cheese to which penicillin was added.

TABLE 1

Lactic acid production by dairy cultures in preparations containing mold ripened cheese with and without added penicillin

Sample number	Mold-ripened cheeses		Concentration of cheese in preparation		
			2	2	10
			Units[a] penicillin added		
			0.2	0	0
	Variety	pH	Titratable acidities after 6 hours[b] incubation at 30° C.		
			(%)	(%)	(%)
1	Blue	6.55	0.18	0.74	0.77
2	Blue	5.41	0.17	0.72	0.79
3	Blue	5.51	0.20	0.63	0.66
4	Blue	5.56	0.16	0.67	0.69
5	Blue	5.87	0.17	0.61	0.67
6	Roquefort	6.05	0.19	0.62	0.63
7	Roquefort	6.85	0.23	0.60	0.73
8	Roquefort	6.95	0.22	0.63	0.67
9	Camembert	7.87	0.18	0.65	0.77
10	Camembert	7.80	0.20	0.64	0.68
11	Camembert	7.85	0.19	0.65	0.68
12	Nuworld	6.75	0.17	0.71	0.74
13	Nuworld	5.60	0.19	0.68	0.71
14	Nuworld	5.90	0.20	0.62	0.69

[a] Units per gram final preparation.
[b] Initial titratable acidity of all samples 0.15 to 0.18% as lactic acid.

at levels of 1.0 and 0.2 units per gram of preparation. At a level of 0.1 unit per gram there was a retarding effect, but at a level of 0.01 unit per gram there was no effect. These results are in agreement with those reported earlier by Krienke *(10)* that the critical concentration of penicillin against lactic streptococci is about 0.1 unit per gram.

Shown in Table 1 are results pertaining to two concentrations of the 14 samples of mold-ripened cheeses tested. As significant differences were apparent at the end of 6 hours of incubation at 30° C., the titratable acidities were compared after such a period of time using the values obtained when 0.2 unit of penicillin was present. As the penicillin was added to the cheese preparations at the pH level shown in Table 1, it was evident that such acidities did not inactivate penicillin under these experimental conditions, although Baron *(2)* reported that penicillin is unstable in aqueous solution and is decomposed rapidly by acids, alkalies, and penicillinase.

It may be noted that in the presence of 0.2 unit of penicillin the initial titratable acidities in some instances tended to increase slightly during the 6-hour incubation period. In the penicillin-free samples the acid development was above 0.6% after 6 hours of incubation. This is to be expected with active starters. The data for sample No. 6 (Table 1) are a portion of those used in preparing Figures 1 and 2.

If there had been as much as 2.0 units of penicillin per gram in any sample of mold-ripened cheese, the final concentration of penicillin, when 10% cheese was used in the preparations, would have been 0.2 unit per gram, which would

have resulted in complete inhibition of lactic acid development, and if the original cheese had contained 1.0 unit of penicillin per gram, a reduction in the rate of acid development would have been observed by this method of penicillin assay. Thus, it may be concluded that none of the cheeses tested for penicillin contained as much as 2.0 units of penicillin per gram of cheese, which was the limit of sensitivity of the method used. For all practical purposes it may be assumed that there was no penicillin present in the samples of mold-ripened cheeses assayed. On the same basis, it can be assumed that no other antibiotic known for its inhibitory effect on dairy starters was present.

SUMMARY

A study was made to determine whether antibiotics are present in mold-ripened cheeses. The assay method used relied upon the titratable acidities, calculated as lactic acid, produced by regular commercial dairy cultures. The suitability and accuracy of the method was tested by additions of known quantities of penicillin to preparations containing mold-ripened cheese. The method was sensitive to a concentration of 2.0 units of penicillin per gram of cheese.

Fourteen different commercial brands of mold-ripened cheeses were assayed. The samples of cheese included five Blue, three Roquefort, three Camembert, and three Nuworld. One brand of Blue and three brands of Roquefort were imported (Denmark and France, respectively), and the remainder were manufactured in the United States.

None of the samples of cheese tested contained as much as 2.0 units of penicillin per gram of cheese. No other culture inhibitory substances were present in the cheese in such concentration that they could be detected when as much as 10% cheese was included in the culture preparations assayed. For all practical purposes it may be concluded that there is no penicillin present in commercial Blue, Roquefort, Camembert, or Nuworld cheese.

ACKNOWLEDGMENTS

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REFERENCES

(1) Backus, M. P., Stauffer, J. F., and Johnson, M. J. Penicillin Yields from New Mold Strains. *J. Am. Chem. Soc.*, **68**: 152. 1946.

(2) Baron, A. L. *Handbook of Antibiotics.* Reinhold Publishing Corp., New York. 1950.

(3) Calbert, H. E. The Problem of Antibiotics in Milk. *J. Milk and Food Technol.*, **14**: 61. 1951.

(4) Claybaugh, G. A., and Nelson, F. E. The Effect of Antibiotics in Milk: A Review. *J. Milk and Food Technol.*, **14**: 155. 1951.

(5) Committee on Antibiotics in Milk. *Mimeo. report* presented at the 46th Annual Meeting of A. D. S. A., Knoxville, Tenn., June 8, 1951.

(6) COMMITTEE ON ANTIBIOTICS IN MILK. *Mimeo report* presented at the 48th Annual Meeting of A. D. S. A., Madison, Wis., June 23, 1953.
(7) FOOD AND DRUG ADMINISTRATION. *Federal Register*, **18**: 1077. 1953.
(8) KATZNELSON, H., AND HOOD, E. G. Influence of Penicillin and Other Antibiotics on Lactic Streptococci in Starter Cultures Used in Cheddar Cheesemaking. *J. Dairy Sci.*, **32**: 961. 1949.
(9) KNIGHT, S. G., MOHR, W. H., AND FRAZIER, W. C. White Mutants of *Penicillium roqueforti*. *J. Dairy Sci.*, **33**: 929. 1950.
10) KRIENKE, W. A. Penicillin in Milk; A Hazard to Starters, Buttermilk and Cottage Cheese Manufacture. *Milk Dealer*, **39** (2): 126. 1949.
11) MORRIS, H. A., JEZESKI, J. J., AND COMBS, W. B. The Use of White Mutants of *Penicillium roqueforti* in Cheese Making. *J. Dairy Sci.*, **37**: 711. 1954.
12) RAPER, K. B., AND THOM, C. *A Manual on the Penicillia*. The Williams and Wilkins Co., Baltimore, Md. 1949.
13) TROUT, G. M. Dairy Technology Research in the United States from 1949 to 1952, inclusive — Part I. *Dairy Sci. Abstr.*, **15**: 241. 1953.

B-VITAMIN LEVELS IN THE BLOOD OF YOUNG DAIRY CALVES FED A MILK REPLACEMENT DIET WITH AND WITHOUT AUREOMYCIN[1,2]

Q. T. SMITH AND R. S. ALLEN
Department of Chemistry, Iowa State College, Ames

The mechanism by which antibiotics exert a growth-stimulating effect in chicks, pigs, and calves has not been definitely established. Jukes and Williams *(9)* have reviewed the various hypotheses proposed to account for the mode of action of antibiotics, and the basis for most of the proposals is the assumption that the antibiotics exert an influence on the intestinal microflora.

Since it has been established *(10)* that certain B-complex vitamins are synthesized in the intestinal tract of young calves on a limited whole milk regime, it seems that an antibiotic possibly may alter the intestinal microflora in a manner such that greater or less than normal quantities of B-vitamins may be present in the intestinal tract. The availability to the calf of intestinally synthesized vitamins has not been adequately determined, but it is generally assumed that part of these vitamins may be absorbed. Whether blood levels of the B-vitamins in calves are correlated with absorption and/or state of metabolism of these vitamins has not been ascertained.

The objectives of the present study were to determine the blood levels of thiamine, riboflavin, niacin, pantothenic acid, and vitamin B_{12} activity in Holstein calves at 4 days of age and to ascertain the influence of orally fed aureomycin on the blood levels of these B-vitamins in Holstein calves on a whole milk replacement feeding regime for a period of 12 weeks.

EXPERIMENTAL PROCEDURE

Two groups of Holstein calves were selected for this study. Calves of one group (ten males and ten females) from the Iowa State College dairy herd were allowed to remain with their respective dams for 3 days following birth. To characterize the early postnatal blood B-vitamin levels, samples of venous blood (potassium oxalate anticoagulant) were drawn on the fourth day and analyzed as described below for thiamine, riboflavin, pantothenic acid, niacin, and vitamin B_{12}.

A second group of eight Holstein calves (four males and four females) at 4 days of age were placed on a milk replacement feeding program. The milk replacement, containing primarily dried whey product reconstituted with water (14% dried whey product, 86% water), was fed for the first 7 weeks at the following daily rates per 100 lb. body weight: 3.0, 5.4, 10, 8, 8, 6, and 4 lb.,

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[2] Supported in part through funds provided by Western Condensing Co., Appleton, Wis.

respectively. Whole milk averaging 3% fat was fed at the daily rates of 5.0 lb. and 3.6 lb. per 100 lb. body weight during the first and second weeks on experiment. No milk or milk replacement was fed subsequent to 7 weeks. A calf starter containing 40% ground corn, 20% crushed oats, 28% soybean oil meal, 10% wheat bran, 1% steamed bone meal, and 1% iodized salt was fed ad libitum until a maximum of 4 lb. was consumed daily per calf. Medium quality mixed hay, largely alfalfa and brome grass, was fed free choice. Four calves, two males and two females, served as controls while the other four animals in this group were fed the same ration plus aureomycin.[3] The antibiotic was fed to each animal at the daily rate of 40 mg. of aureomycin via the liquid portion of the diet during the first 7 weeks and at the daily rate of 80 mg. in the concentrate mixture from 8 to 12 weeks on experiment. With a few exceptions, venous blood samples were drawn at the start of the experiment and at 1, 2, 4, and 8 weeks thereafter. In addition, several samples were taken at 12 weeks.

After enzymatic hydrolysis of the blood with a combination of clarase and papain, riboflavin, pantothenic acid, and niacin were determined microbiologically with *Lactobacillus casei* by a modification of the method of Clegg, Kodicek, and Mistry *(5)*. The thiochrome method *(2)* was employed for measuring blood thiamine values. Extraction of vitamin B_{12} activity from the blood involved heating the sample to which cyanide had been added, mixing with water in a Waring blendor, and filtering. The vitamin B_{12} activity was estimated with *Lactobacillus leichmannii* by a procedure based upon the method of the United States Pharmacopoeia *(23)*.

TABLE 1

Mean blood B-vitamin levels in 4-day-old Holstein calves (10 male, 10 female)

Vitamin	Male	Female
Thiamine *(γ/ml)*	0.074 ± 0.011[a]	0.069 ± 0.010[b]
Riboflavin *(γ/ml)*	0.21 ± 0.03	0.23 ± 0.03
Pantothenic acid *(γ/ml)*	1.91 ± 0.33	2.04 ± 0.44
Niacin *(γ/ml)*	9.96 ± 3.4	12.1 ± 2.9
Vitamin B_{12} activity *(mγ/ml)*	0.90 ± 0.20	0.94 ± 0.16[b]

[a] Mean value ± standard error.
[b] Values for 8 calves only.

RESULTS

The blood B-vitamin levels in 4-day-old Holstein calves are summarized in Table 1. No statistically significant differences between male and female animals were found in the vitamins studied. The variations in values for each vitamin, however, were great.

The average blood B-vitamin values for calves fed the basal diet with and without aureomycin are presented in Figures 1 and 2. Only the calves for which all the vitamin values within an age period were determined are included

[3] As Aurofac D supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

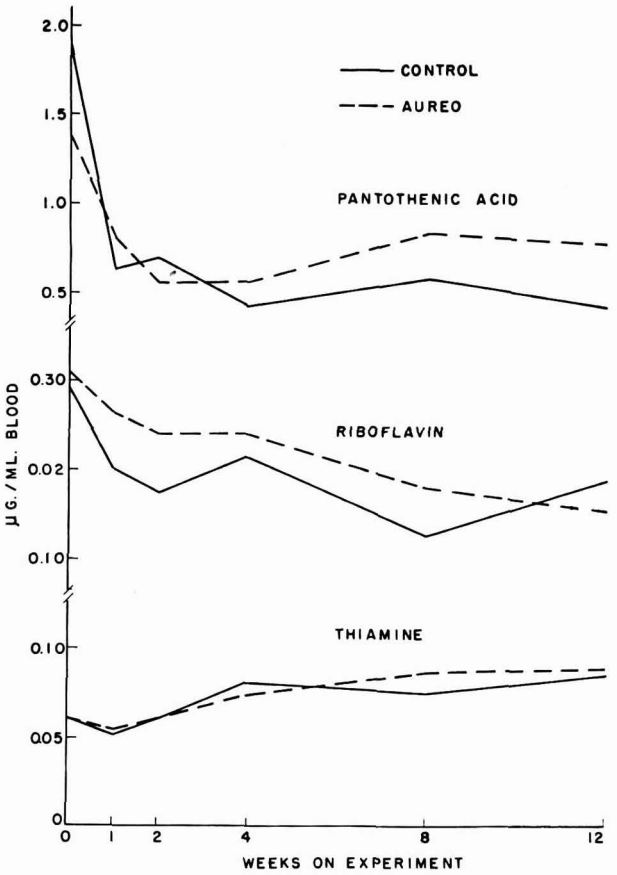

FIG. 1. Changes in the blood pantothenic acid, riboflavin, and thiamine values in control and aureomycin-supplemented dairy calves.

in these graphs. The values at 12 weeks represent only two calves, whereas the other values are averages for three. Missing plot estimations were employed to supply single values for the aureomycin-supplemented group at 2 weeks (thiamine), 4 weeks (vitamin B_{12}), and 12 weeks (vitamin B_{12}). There were no statistically significant differences between the aureomycin-supplemented group and the control group at any age. Also, there were no apparent differences between male and female animals.

Since the differences between experimental groups were not significant, all of the available blood B-vitamin data for the eight calves at various ages were combined and summarized in Table 2. The average vitamin levels appear in certain cases to change with age. Since the values at various ages include a variable number of determinations, differences between various ages were tested by comparing values for calves from which samples were obtained at both age-

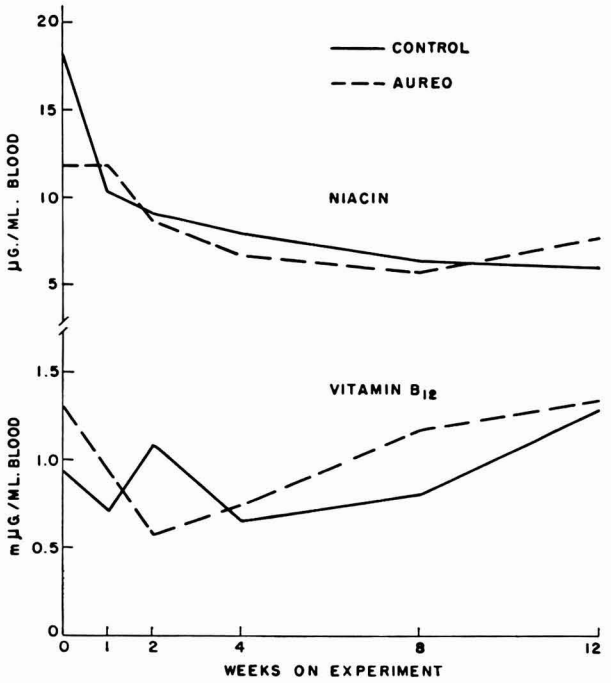

FIG. 2. Changes in the blood niacin values and vitamin B_{12} activity in control and aureomycin-supplemented dairy calves.

periods in question. By this procedure it was found that during the first week on experiment the decreases in pantothenic acid and thiamine levels were significant at the $P = 0.05$ level, and the niacin and vitamin B_{12} activity changes approached significance ($P = 0.1$). The decline in riboflavin values during the same period was not significant, but from 4 to 8 weeks the drop in blood riboflavin values was significant at the $P = 0.05$ level. Although other trends in the blood B-vitamins at several intervals were observed, none was significant at the $P = 0.05$ level.

The aureomycin-supplemented animals grew at an accelerated rate. This observation is in accordance with previous reports *(11, 14)*.

DISCUSSION

The variability of the blood B-vitamin values, except for vitamin B_{12}, was considerably greater in calves at 4 days of age than that observed at later age periods. These data suggest possible differences in the state of nutrition of the calves due, at least in part, to the levels of the B-vitamins in the colostrum of the dam. Another factor which may be involved is the total quantity of nutrients consumed during the colostral period. Calves on experiment subsequent to

TABLE 2

B-vitamin levels for Holstein calves at various ages

Weeks on expt.	No. calves	Thiamine	Riboflavin	Pantothenic acid	Niacin	Vitamin B_{12} activity
			(γ/ml)			*(mγ/ml)*
0	6	0.062 ± 0.008[a]	0.28 ± 0.05	1.61 ± 0.38	15.1 ± 5.5	1.11 ± 0.18
1	8	0.057 ± 0.007	0.22 ± 0.03	0.80 ± 0.16	12.0 ± 1.9	0.73 ± 0.14
2	8	0.062 ± 0.007(6)[b]	0.21 ± 0.02	0.69 ± 0.12	8.6 ± 1.1	0.80 ± 0.18(7)
4	6	0.079 ± 0.006(5)	0.23 ± 0.02	0.49 ± 0.06	7.3 ± 0.8	0.62 ± 0.14(5)
8	6	0.081 ± 0.005	0.15 ± 0.02	0.72 ± 0.10	6.1 ± 0.2	1.00 ± 0.32
12	5	0.086 ± 0.007	0.17 ± 0.03	0.60 ± 0.11	7.1 ± 0.6	1.33 ± 0.25(3)

[a] Mean values ± standard error.

[b] Numbers in parentheses denote number of calves when different from column 2.

4 days of age received approximately equal quantities of feed, and the variability in blood B-vitamin values decreased.

The data presented in this report indicate that the feeding of aureomycin had no marked effect on the blood levels of thiamine, riboflavin, pantothenic acid, niacin, and vitamin B_{12}. These results fail to explain an earlier observation by Murley *(13)* wherein an apparent riboflavin deficiency occurred in one young calf and a thiamine deficiency in another, both of which were receiving aureomycin in the diet. It now seems probable that factors other than antibiotic supplementation per se were responsible for the apparent B-vitamin deficiencies observed by Murley.

Evidence that indicates gastro-intestinal synthesis of B-vitamins in ruminants is available. Kesler and Knodt *(10)* found that on a dry matter basis the concentrations of thiamine, riboflavin, niacin, and pteroylglutamic acid were higher in various regions in the digestive tract of the young dairy calves than in the feed consumed. Further evidence of intestinal synthesis of some of the B-vitamins has been reported by Pearson *et al. (16)* in studies with sheep. It is of interest to note that Chance *et al. (3)*, in a study of the effect of aureomycin on the rumen synthesis of some of the B-vitamins, found that the antibiotic appeared to have no marked effect on the rumen synthesis of riboflavin, pantothenic acid, or nicotinic acid.

The effect of antibiotics on the intestinal synthesis of some of the B-vitamins by various species has received attention during the past few years. Several reports *(4, 8, 17)* have shown that vitamin B_{12} is synthesized in the intestines of rats and that aureomycin feeding results in an increase in the intestinal level of this vitamin. The oral administration of streptomycin to humans apparently has no marked effect on the urinary excretion of folic acid, thiamine, riboflavin, and pyridoxine compounds *(19)*. This finding suggests that streptomycin did not increase intestinal synthesis of the vitamins studied. Sauberlich *(20)* has shown, however, that the addition of penicillin to a diet caused a marked stimulation in the growth of rats fed diets free of or low in thiamine, pyridoxine, and pantothenic acid. The inclusion of penicillin or aureomycin in the complete diet had no effect upon the growth of the animals. The recent report by Guggenheim *et al. (7)* indicates that aureomycin, streptomycin, and terramycin added to diets low in pantothenic acid cause a significant increase in the fecal excretion of this vitamin in the rat, and also that the antibiotics caused increased urinary excretion of thiamine and pantothenic acid at all levels of vitamin intake. It seems apparent, therefore, that one might expect little adverse effect, and quite probably a beneficial effect, of aureomycin-supplementation on the intestinal synthesis of several of the B-complex vitamins in most species.

The blood B-vitamin levels reported herein are within the range of the values reported for bovine blood levels of riboflavin *(6, 21)*, niacin *(15, 22)*, thiamine *(6, 24)*, and vitamin B_{12} activity *(1, 18)*. The authors are not aware of reports of blood pantothenic acid values in young dairy calves. Moreover, virtually no data are available to show the trends in blood levels of the B-vitamins during the early life of the young calf. A recent report by Moinuddin *et al. (12)* indicates

that the levels of both riboflavin and niacin in lambs' blood decrease gradually from birth to 6 weeks, whereas the blood levels of vitamin B_{12} tend to increase slightly from birth to 8 weeks. Similar trends were observed in calves in the present study.

The observed trends in the blood B-vitamin values cannot be explained solely by gradual change in the feeding regime over the 12-week experimental period. However, the significant drop in the riboflavin level during the 4-to-8-week period may be due in part to the discontinuation of whey product (high in riboflavin) feeding and the subsequent consumption of hay and grain (low in riboflavin) during this period. The onset of rumination may be an important factor in the observed changes in blood levels of vitamins.

Since the urinary excretions of the B-vitamins under consideration in this report were not measured, one cannot state that the B-vitamin absorption from the intestinal tract was not influenced by the oral administration of aureomycin. However, since the blood B-vitamin levels were essentially the same in each group of calves, it is apparent that the antibiotic had no demonstrable adverse effect. Additional studies, which should include urinary excretions of the B-vitamins, are needed to clarify the over-all problem of B-vitamin metabolism in calves receiving antibiotics.

SUMMARY

The blood levels of thiamine, riboflavin, pantothenic acid, niacin, and vitamin B_{12} activity were determined for ten male and ten female Holstein calves at 4 days of age. No significant differences between sexes were observed.

Eight Holstein calves at 4 days of age were placed on a milk replacement diet and were assigned to two comparable groups, one of which received aureomycin orally (40 mg. daily per calf for 7 weeks, 80 mg. daily per calf from 8 to 12 weeks) while the other served as a control. Venous blood samples drawn at the beginning of the experiment and at 1, 2, 4, 8, and 12 weeks thereafter were analyzed for thiamine, riboflavin, niacin, pantothenic acid, and vitamin B_{12}. No significant differences in the blood levels of these vitamins were found between the two groups of animals. Moreover, no apparent differences were observed between male and female calves. Certain trends in the B-vitamin blood levels with age are evident.

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REFERENCES

(1) Anthony, W. B., Rupel, I. W., and Couch, J. R. Vitamin B_{12} in Blood of Mature Dairy Cows Liberally Fed Grain, Alfalfa Hay, Silage, and Pasture Forage as Measured by *Lactobacillus leichmannii* 4797 (ATCC). *J. Dairy Sci.*, **34**: 295. 1950.

(2) Association of Vitamin Chemists, Inc. *Methods of Vitamin Assay*, 2nd ed. Interscience Publishers, Inc., New York. 1951.

(3) CHANCE, C. M., DUNCAN, C. W., HUFFMAN, C. F., AND LUECKE, R. W. Antibiotics in Rumen Digestion and Synthesis. II. The Effect of Aureomycin on the Concentration of Some Amino Acids and B Vitamins in the Rumen. *J. Dairy Sci.*, **36**: 495. 1953.

(4) CHOW, B. F., DAVIS, R. L., AND DAVIS, S. The Effect of Antibiotics and the Composition of Diets on Fecal Vitamin B_{12} and Biotin. *J. Nutrition*, **49**: 657. 1953.

(5) CLEGG, K. M., KODICEK, E., AND MISTRY, S. P. A Modified Medium for *Lactobacillus casei* for the Assay of B Vitamins. *Biochem. J.*, **50**: 326. 1951.

(6) FUJITA, A., AND YAMADORI, M. Distribution of Thiamine and Riboflavin in Components of Blood. *Arch. Biochem.*, **28**: 94. 1950.

(7) GUGGENHEIM, K., HALEVY, S., HARTMANN, I., AND ZAMIR, R. The Effect of Antibiotics on the Metabolism of Certain B Vitamins. *J. Nutrition*, **50**: 245. 1953.

(8) JOHANSSON, K. R., PETERSON, G. E., AND DICK, E. C. Effects of Dietary Aureomycin upon the Intestinal Microflora and the Intestinal Synthesis of Vitamin B_{12} in the Rat. *J. Nutrition*, **49**: 135. 1953.

(9) JUKES, T. H., AND WILLIAMS, W. L. Nutritional Effects of Antibiotics. *Pharmacol. Revs.*, **5**: 381. 1953.

(10) KESLER, E. M., AND KNODT, C. B. Increase in B-Vitamin Concentrations in Digestive Tract Contents of Young Dairy Calves. *Proc. Soc. Exptl. Biol. Med.*, **79**: 85. 1952.

(11) LOOSLI, J. K., WASSERMAN, R. H., AND GALL, L. S. Antibiotic Studies with Dairy Calves. *J. Dairy Sci.*, **34**: 500. 1951.

(12) MOINUDDIN, M., POPE, A. L., PHILLIPS, P. H., AND BOHSTEDT, G. The Effect of Ration on the Riboflavin, Niacin, and Vitamin B_{12} Concentration of the Blood and Milk of Sheep. *J. Animal Sci.*, **12**: 497. 1953.

(13) MURLEY, W. R. Unpublished Ph.D. Thesis, Iowa State College Library, Ames. 1952.

(14) MURLEY, W. R., JACOBSON, N. L., AND ALLEN, R. S. The Effect of Aureomycin Supplementation on Growth and Feed Utilization of Young Dairy Calves. *J. Dairy Sci.*, **35**: 846. 1952.

(15) NIEMEIJAR, J. A. Presence of Nicotinic Acid (Free and Combined) in Blood. *Rec. trav. chim.*, **65**: 84. 1946.

(16) PEARSON, P. B., STRUGLIA, L., AND LINDAHL, I. L. The Fecal and Urinary Excretion of Certain B Vitamins by Sheep Fed Hay and Semi-Synthetic Rations. *J. Animal Sci.*, **12**: 213. 1953.

(17) PETERSON, G. E., DICK, E. C., AND JOHANSSON, K. R. Influence of Dietary Aureomycin and Carbohydrate on Growth, Intestinal Microflora and Vitamin B_{12} Synthesis of the Rat. *J. Nutrition*, **51**: 171. 1953.

(18) PFANDER, W. H., DIETRICH, L. S., MONSON, W. J., HARPER, A. E., AND ELVEHJEM, C. A. Citrovorum Factor, Vitamin B_{12} and Folic Acid Activity of the Whole Blood of Several Species. *Proc. Soc. Exptl. Biol. Med.*, **79**: 219. 1952.

(19) SARETT, H. P. Effect of Oral Administration of Streptomycin on Urinary Excretion of B Vitamins in Man. *J. Nutrition*, **47**: 275. 1952.

(20) SAUBERLICH, H. E. Effect of Aureomycin and Penicillin upon the Vitamin Requirements of the Rat. *J. Nutrition*, **46**: 99. 1952.

(21) STRONG, F. M., FEENEY, R. E., MOORE, B., AND PARSONS, H. T. The Riboflavin Content of Blood and Urine. *J. Biol. Chem.*, **137**: 363. 1941.

(22) TUOMAINEN, L. Effect of Nicotinic Acid on the Blood of the Cow and the Nicotinic Acid Percentage of the Milk. *Suomen Kemistilehti*, **23**: 203. 1950. (Abstracted in *Chem. Abstr.*, 7211a. 1951).

(23) U. S. PHARMACOPOEIA, 14th Rev., Suppl. 3: 15. 1951.

(24) WESTENBRINK, H. G. K., PARVE, E. P. S., LINDER, A. C. VANDER, AND BROCK, W. A. Determination of Cocarboxylase in Blood. *Z. Vitaminforsch.*, **13**: 218. 1943.

METHODS-TIME MEASUREMENT ANALYSIS OF SOME MILK PLANT CLEANING OPERATIONS[1]

G. P. MARLEY, W. M. ROBERTS, AND R. W. LLEWELLYN
Departments of Animal Industry and Industrial Engineering
North Carolina State College, Raleigh

There are many methods used to perform the task of disassembling, cleaning, and reassembling dairy equipment among different plants as well as within individual plants. The wide variations in methods depend to some extent on the plant layout and equipment available to do the job but to a greater extent on the training, experience, skill, and incentive of the operator. With the exception of the sanitary lines operations, most of the disassembly, cleaning, and assembly operations occur only once each day, thus reducing the worker's desire to perform the operation the same way twice in succession.

Several reports on the processing cost in dairy plants have revealed that labor is the greatest single item of expense. These reports also indicate that dairy plant clean-up accounts for a substantial portion of the labor cost. Therefore, the application of a technique which would improve and standardize the methods of cleaning any given piece of equipment used in the processing of milk would seem to be beneficial in reducing the total labor cost.

It would be advantageous for plant supervisory personnel to know how long it should take to do a given clean-up task. Such knowledge would lead to better use of available manpower, provide a basis for judging efficiency, help in estimating labor requirements and workload distribution and, perhaps at a later date, aid in the formulation of wage incentive payment procedure.

Previous studies of dairy plant activities have been reported in which times were obtained by stopwatch time study. This technique has some disadvantages because *(a)* the methods and layout vary so much from plant to plant that time differentials are encountered from one study to the next which are difficult to account for because of the many variables involved; *(b)* only one reading can be obtained per day, thus making the process of accumulating data very slow; and *(c)* a complete study must be made on each make and size of machine before standard data can be established for the machine.

Methods-time measurement (MTM) is a relatively new technique and was used in this study to overcome the disadvantages mentioned above. MTM involves the determination of methods and times from a table of standard time values for the fundamental body motions used in industrial work *(1)*. The principal motions are reach, move, turn, grasp, position, disengage, and release. The first two motions, reach and move, are classified by type and the distance the hand

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moves in performing the motion; turn is classified by type and the number of degrees the wrist turns during the turning motion; the others are classified by type. For the classification of each motion, a time value is given. To establish a time standard for an operation, it is only necessary to select the motions required to perform the task from the standard table and add the standard time values for the motions involved.

The three objectives in this study were: *(a)* to develop more efficient methods of performing cleaning operations; *(b)* to establish standard times for the methods selected; and *(c)* to evaluate the practicability of the methods and the accuracy of the standard times when subjected to plant conditions.

METHODS

Several milk plants in North Carolina were visited to observe the cleaning methods now being used. The plants ranged in size from 5,000 to 70,000 lb. of milk received daily. For a period of 8 weeks data were collected on the clean-up operations for the following equipment: homogenizers,[2] A200, B500, and B800; glass bottle fillers, A10 and C14; and HTST, D2000. In general, the following procedure was used regardless of the make or size of equipment under study. The instruction book for the machine under observation was first studied to become familiar with the nomenclature of the individual parts. As the operator disassembled, washed, and assembled the machine, notes were taken on plant arrangement, accessibility of equipment to cleaning, the use (and possible use) of cleaning aids such as portable wash carts and vats, length and type of motions

TABLE 1

Example of the MTM technique for establishing standard times for element "loosen nuts by hand and aside to pail"

Left hand	Symbol	TMU[a]	Symbol	Right hand
Hand to nut	R20B	18.6	R20B	Hand to nut
Grasp nut	GIA	1.7	GIA	Grasp nut
Turn nut to loosen	T90S	5.4	T90S	Turn to loosen
Release nut	RLI	1.7	RLI	Release nut
To new position on nut	T90S	5.4	T90S	To new position on nut
Grasp nut	GIA	1.7	GIA	Grasp nut
Turn nut to loosen	T90S	5.4	T90S	Turn nut to loosen
Release nut	RLI	1.7	RLI	Release nut
		42.6		Repeat last four motions three times
Regrasp nut	G2	5.6	G2	Regrasp nut
Nut to pail	M20C	18.6	M20C	Nut to pail
Release nut to pail	RLI	1.7	RLI	Release nut to pail
		1101.0		Repeat last 20 motions ten times for remaining 20 nuts
		1211.1 × 0.0006[b] = 0.73 minute		

[a] Time measurement units — one TMU is defined as 0.00001 hr.
[b] Factor for converting TMU's to decimal minutes.

[2] The letter is the code designation for the manufacturer of each piece of equipment. The numerals indicate the capacity of the equipment as follows: homogenizers, gal/hr; bottle fillers, number of valves; and HTST, lb/hr.

involved, and number of steps necessary to do the job. The best method of performing the operation was then developed with the MTM technique, and the time for the operation was calculated.

A typical example of the MTM technique for establishing standard times is given in Table 1.

The complete data including all the individual motions and their times for each element are filed in the Dairy Manufacturing Section of the Animal Industry Department, North Carolina State College, Raleigh.

The second phase of the study was directed at establishing the practicability of the MTM data when applied to plant conditions. A stopwatch time and motion study was used for this purpose. The methods which had been calculated by the MTM study to be the most efficient were taught to experienced operators on the following pieces of equipment; A200 homogenizer, A10 bottle filler, and D2000 HTST pasteurizer. After a training period of 1 to 2 weeks, stopwatch readings were determined for each element. Ten readings for each element were taken, and the arithmetical average of these was determined.

RESULTS AND DISCUSSION

Standard times were established for the preparation, disassembly, washing, and assembly of the homogenizer, glass bottle filler, and HTST pasteurizer by the stopwatch and MTM techniques.

Homogenizer operation. A comparison of standard times as established by stopwatch and MTM for the disassembly, wash, and assembly of the A200 homogenizer is given in Table 2.

TABLE 2

A comparison of standard times for homogenizer clean-up as established by stopwatch and MTM techniques

Operations	Stopwatch[a] time	MTM time
	(Min.)	*(Min.)*
Preparation	0.65	0.71
Disassembly	3.56	3.37
Wash and rinse	4.60	4.81
Assembly	7.50	6.79
Total clean-up time	16.31	16.04

[a] Average of 10 readings.

The homogenizer was located in a position to give the operator free access to the front and both sides of the machine for efficient operation as well as ease of cleaning. The manufacturer's directions of following the last run of milk with warm water until the discharge becomes clear were observed in this operation. This machine was disassembled into 77 parts, of which 22 were nuts. The parts were placed directly into a rubber pail of cleaning solution as they were disassembled. The milk contact surfaces of all disassembled parts and cylinder block were scrubbed with stiff bristled brushes. As the parts were washed, they

were placed in an orderly arrangement on a rubber mat that was located directly in front of the machine. This eliminated the practice of searching through a pile of jumbled parts for the desired one. After the parts had been rinsed and allowed to dry for 10 to 15 minutes, they were assembled with the exception of the plunger assemblies and front port covers. Management had adopted this policy to assure complete drying of the parts, especially gaskets, and thus increase the life of the machine. Immediately before the next operation, the plunger assemblies and front port covers were assembled.

In previous work, Redfern *(3)* found that it required 23.22 minutes to disassemble, clean, and assemble an A200 homogenizer when the operator was allowed to use his own cleaning method. In this investigation, the same homogenizer was studied. No change or improvements were made except in methods used to perform the clean-up task. With the improved method, the operator was able to complete the clean-up task in 16.31 minutes, which is a saving of 6.91 minutes, or 42%. In one year approximately 35.5 hours would be saved in clean-up time alone on the homogenizer just by changing the operator's method of performing the task.

From the data it would seem that a more efficient method of disassembling, cleaning, and assembling a homogenizer was developed. The stopwatch and MTM standard times have variations of only 0.27 minute, or 2% for the total clean-up time.

An MTM study was also made on B500 and B800 homogenizers. The results on these machines along with the MTM data for the A200 homogenizer are given in Table 3.

TABLE 3

A comparison of the time required by operation on homogenizer disassembly, wash, and assembly as established by MTM for three machines of different capacities

Operations	A200	B500	B800
	(Min.)	*(Min.)*	*(Min.)*
Preparation	0.71	2.16	2.16
Disassembly	3.73	4.77	4.51
Wash and rinse	4.81	3.46	3.46
Assembly	6.79	7.42	6.17
Total clean-up time	16.04	17.81	16.29

The same general procedures that were used for the A200 machine were followed in cleaning the B500 and B800 machines. It was necessary to use a larger cleaning vat and table, which accounts for the longer preparation time for the B500 and B800 machines. Regardless of the size of the machine, the total clean-up time is approximately the same. The number of parts per machine seems to be the reason for this small variation. The machines were disassembled into 77, 75, and 73 parts for the 200, 500, and 800-gal. machines, respectively. The average times per part were 0.21, 0.24, and 0.22 minute for the 200, 500, and 800-gal. machines, respectively. The smallest machine can be disassembled, washed, and assembled in less time than the larger machines when the time is calculated on a per part basis.

Bottle filler operation. MTM and stopwatch studies were made on the A10 bottle filler. A portable two-compartment wash vat was moved near the bottle filler to enable the operator to disassemble the machine and place the parts directly into the vat. Buttermilk was the last product bottled before clean-up, which made it necessary to do a thorough prerinse of all parts before washing. All parts coming in contact with the milk as well as the exhauster assembly were disassembled. The bowl cover and exhauster hood and tubes were placed on rubber mats near the machine. All disassembled parts except the bowl cover and exhauster hood and tubes were washed in the vat. The above mentioned parts plus all stationary parts of the bottle filler and capper were washed from a pail of cleaning solution. After being rinsed with warm and then hot water, the machine was allowed to dry 10 to 15 minutes before assembly. The machine was completely assembled at the end of the day with the exception of the condensate deflectors, valve rubbers, transfer guide, and starwheels. These parts were assembled immediately before the next operation.

A comparison of the standard times as established by the stopwatch and MTM technique for the bottle filler clean-up is given in Table 4. The times established by stopwatch and MTM were 29.49 and 28.19 minutes, respectively, for the total cleaning time. This is a variation of 1.30 minutes, or 5%, which is as close as can be expected for this type of nonrepetitive operation.

TABLE 4

A comparison of standard times as established by the stopwatch and MTM technique for the disassembly, wash, and assembly of a bottle filler

Operations	Stopwatch[a] time	MTM time
	(Min.)	*(Min.)*
Preparation	0.76	0.56
Disassembly	3.99	4.06
Prerinse and prepare cleaning solution	3.40	3.40[b]
Wash and rinse	11.60	11.57
Assemble	9.74	8.60
Total clean-up time	29.49	28.19

[a] Average of 10 readings.
[b] Stopwatch time.

Redfern *(2)* found that it required 47.34 minutes to disassemble, clean, and assemble an A10 bottle filler. In this investigation, an improved method was established by the MTM technique for the same bottle filler. With the improved method, the operator was able to clean the machine in 29.49 minutes. The reduction in time of 17.85 minutes, or 61%, would mean a saving of approximately 93 hours a year in clean-up time for the bottle filler.

The general cleaning procedure for the C14 machine was the same as for the A10 machine. The only difference was that the capper was cleaned separately on the C14 machine and therefore was not included in the study. Table 5 gives a comparison of the time required to clean the two machines.

The C14 bottle filler can be disassembled, cleaned, and assembled in 23%

TABLE 5

A comparison of the time required by operation on bottle filler disassembly, wash, and assembly as established by MTM for two machines of comparable size

Operations	A10	C14
	(Min.)	*(Min.)*
Preparation	0.56	0.70
Disassembly	4.06	2.36
Prerinse and prepare cleaning solution	3.40[a]	3.40[a]
Wash and rinse	11.57	11.72
Assemble	8.60	4.69
Total clean-up time	28.19	22.87

[a] Stopwatch time.

less time than the A10 machine. When the total clean-up times are compared on a per part disassembled basis, however, it requires 0.01 minute longer per part to clean the C14 machine. The A10 machine was disassembled into 93 parts, of which 70 were filling valve assemblies, and the C14 machine was disassembled into 73 parts, of which 56 were filling valve assemblies.

HTST pasteurization operation. The problem of establishing standard data on the high-temperature short-time pasteurization system is very complex because of the many different pieces of equipment involved. In this study the system was considered as beginning with the supply tank, located near the HTST pasteurizer proper, and ending at the elbow of the pipe leading into the bottle filler. The equipment between these points included the HTST pasteurizer proper (frame, plates, and connections), positive flow pump, holding tube, flow diversion valve, thermometers, thermal control bulb, air relief valve, three-way valve, two-way valve, and all connecting pipes, tees, and elbows.

Standard times as established by the stopwatch and MTM technique for the D2000 HTST pasteurizer are given in Table 6.

The variations between stopwatch and MTM times were greater for the HTST pasteurizer than for the homogenizer or bottle filler. The wider variety of equipment making up the pasteurization system probably had a greater influence on the operator's ability to learn the new method of performing the clean-up

TABLE 6

A comparison of the time required by operations on HTST pasteurizer disassembly, wash, and assembly as established by stopwatch and MTM techniques

Operations	Stopwatch[a] time	MTM time
	(Min.)	*(Min.)*
Change pipe connections from milk setup to acid setup	14.52	15.46
Timing pump	6.80	5.02
Disassemble pipe from acid setup	12.69	8.08
HTST proper	18.82	18.91
Wash pipes	24.12	15.10
Assemble pipes and valves	49.12	29.53
Total clean-up time	126.07	92.10

[a] Average of 10 readings.

job. The two major causes for the variation in total times, however, were the lack of proper supports for the holding tube and the lack of a quick means of identifying the pipe during the assembly operation. No attempt was made in this study to change anything except the operator's method of performing the work; therefore, these two deficiencies were not corrected prior to the stopwatch study. The total clean-up time established by the MTM technique was 37% below the stopwatch time, which means there is still a potential savings in total clean-up time of 34 minutes a day, or approximately 210 hours a year. By correcting the two situations mentioned above and by extending the training period of the operator, it is believed that the MTM standard times can be attained. No previous times were available for comparison with this method of cleaning HTST pasteurizers.

The data on the clean-up operations of the homogenizer, glass-bottle filler, and HTST pasteurizer indicate that the MTM technique does have merit when applied to these particular operations. Before an unequivocal statement can be made, however, concerning the practicability of MTM as a time-setting technique for the above jobs, more data with different operators working under different plant conditions will be required. The data also indicate that MTM can be used advantageously not only in determining methods for the operators to use in performing the clean-up tasks but in pointing out the proper equipment and correct cleaning aids to use so that clean-up can be done efficiently.

CONCLUSIONS

The methods-time measurement standard data are applicable to nonrepetitive clean-up operations in the dairy plant.

The times required for the disassembly, cleaning, and assembly of the homogenizer, bottle filler, and high-temperature short-time pasteurizer were reduced by 40% or more when operators had been trained to use improved methods established by methods-time measurement.

The training and skill of the operator were the major causes for the variations between the stopwatch and methods-time measurement standard times.

For any particular machine, the number of parts disassembled, washed, and assembled is more important in determining the total clean-up time than size of the machine.

Inefficient use of cleaning aids and equipment can be ascertained by methods-time measurement.

REFERENCES

(1) MAYNARD, H. B., STEGEMERTEN, G. T., AND SCHWAB, J. L. *Methods-measurement.* 1st ed. McGraw-Hill Book Company, Inc., New York. 1948.

(2) REDFERN, R. B. A Time and Motion Study of Some Dairy Plant Cleaning Operations. Thesis, North Carolina State College. 1952.

(3) REDFERN, R. B. Personal Communications. Raleigh. 1952.

DILUTERS FOR BOVINE SEMEN. III. EFFECT OF LACTENIN AND OF LACTOPEROXIDASE UPON SPERMATOZOAN LIVABILITY[1]

R. J. FLIPSE, STUART PATTON, AND J. O. ALMQUIST
Dairy Cattle Breeding Research Center, Department of Dairy Husbandry
The Pennsylvania State University, State College

The fractionation of milk proteins by Thacker *et al. (14)* indicated that the factor(s) present in unheated milk which are toxic to bovine spermatozoa are associated with the albumin-containing fractions. However, much of the activity was lost during the fractionation procedure and none of the fractions retained more than a portion of the toxicity of the source material. The heat lability, nondialyzability and loss of activity with salt fractionation suggested that the factor might be enzymatic in nature. Most of the known enzymes in milk are inactivated by heating to 92° C. for 10 minutes, although lactoperoxidase is a notable exception *(9)*. Another approach to the problem of identifying the spermicidal factor(s) is suggested by the similarity between the characteristics of the spermicidal factor(s) of unheated milk *(5, 13, 14)* and lactenin *(1, 2, 6, 7, 8, 17, 18)*, an antistreptococcal substance found in milk which was originally prepared in concentrated form by Jones and Simms *(7)*.

The investigation reported herein was undertaken to further characterize the nature of the spermicidal factor(s) in unheated milk, and to determine if either lactoperoxidase or lactenin accounted for the toxicity of unheated milk to spermatozoa.

EXPERIMENTAL PROCEDURE AND RESULTS

With the exception of one trial in which Ringer-phosphate solution was used, heated skimmilk served as the control diluent and as the vehicle for the prepared fractions throughout the experiment. Fresh, raw skimmilk was obtained from the University dairy plant, heated at 92° C. for 10 minutes, and cooled in running tap water. Twelve to 15 minutes usually was required to reach 92° C. Semen was diluted for storage at the rate of one volume of semen to 50 volumes of diluent. Motility of spermatozoa, after storage of the diluted semen samples at either 4 or 37° C., was estimated at 37° C. by means of a microscope equipped with a thermostage.

Peroxidase. Two lots of peroxidase were prepared from horseradish root as described by Elliott *(3)*. A sample of crystalline lactoperoxidase *(10)* was obtained through the courtesy of B. D. Polis, U. S. Naval Air Development Center, Johnsville, Pa. The peroxidase and lactoperoxidase samples were dialyzed until salt free, then each was adjusted to a nitrogen content of 0.048 mg. of nitrogen per milliliter of solution. Each of these preparations gave positive results when

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qualitatively tested for peroxidase activity. To test the effect of these preparations on spermatozoan survival at 4° C., one volume of peroxidase was added to seven volumes of heated skimmilk and used as the semen diluent. This dilution provided a concentration of enzyme in the diluent equal to the reported level in milk *(9, 10)*. The heated skimmilk used as the control was diluted with distilled water (7:1) in order to maintain a constant milk solids content in all

TABLE 1

Effect of peroxidase from horseradish and from milk on the mean per cent of motile spermatozoa in five ejaculates

	Days of storage at 4° C.							
	1	2	3	4	6	8	10	12
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Heated skimmilk	54	50	46	44	36	10	4	0
Peroxidase, lot 1	60	58	58	52	50	30	12	0
Peroxidase, lot 2	58	58	54	52	46	32	12	0
Lactoperoxidase	60	58	54	46	40	18	0	0

samples. The mean motilities for five ejaculates are shown in Table 1. An analysis of variance *(12)* of the motility observations revealed that all peroxidase treatments were significantly better than the control. Since lactoperoxidase obviously was not spermicidal, attention was directed to lactenin as the source of toxicity.

Lactenin prepared by tryptic digestion. A sample of lactenin was supplied through the courtesy of T. L. McMeekin, Eastern Regional Research Laboratory, Philadelphia, Pa., and tested for spermicidal activity. A preliminary test revealed that the preparation was highly toxic to spermatozoa when added to heated skimmilk at the rate of 10 mg. per milliliter and showed some adverse effect on motility after 4 hours of storage at 37° C. in concentrations as low as 0.4 mg. per milliliter. Based on these findings, a trial was set up in which heated skimmilk containing 5 mg. of lactenin per milliliter was compared with plain heated skimmilk and with unheated skimmilk. The results, presented in Table 2, show that the lactenin preparation was highly toxic to spermatozoa stored at 4° C. The trial was conducted with a single lot of raw skimmilk; in the first replicate of five ejaculates there was no motility after the second day of storage in unheated skimmilk. The diluents were stored at 4° C. in glass vessels until used in the second replicate 2 days later. In the second replicate motility in

TABLE 2

Livability of bovine spermatozoa in milk and in lactenin prepared by tryptic digestion (mean of 10 ejaculates)

	% motile spermatozoa after storage at 4° C. for							
	1 day	2 days	3 days	4 days	6 days	8 days	10 days	12 days
Heated skimmilk	51	37	31	29	25	13	10	6
Unheated skimmilk	32	16	15	12	9	2	0	0
Lactenin	1	0	0					

three ejaculates persisted for 6 days in unheated skimmilk. No difference between replications was noted with either heated skimmilk or the lactenin preparation. Qualitative tests for amylase and for proteolytic activity revealed that both were present in the lactenin sample. Upon inquiry it was found that the sample had been prepared by trypsin digestion and alcohol precipitation *(11)*. Attempts were made to inactivate the trypsin present in the sample with mercuric chloride *(1)* and dialysis to remove the uncombined mercuric chloride but were only partially successful; lactenin so treated was much less toxic than untreated lactenin, and the toxicity after such treatment varied considerably from one trial to another.

Lactenin prepared by acetone precipitation. Lactenin preparation was next attempted by a modification of the acetone fractionation procedure of Auclair and Berridge *(1)*. Whey was obtained from raw skimmilk by rennin action, and the rennin was inactivated by heating at 63° C. for 10 minutes. The whey was cooled to 2° C., and an equal volume of acetone was added in small increments over a 2-hour period while the temperature was gradually reduced to −7° C. The precipitate which formed was recovered by centrifugation in the cold, suspended in ice water, and dialyzed against distilled water at 4° C. for 48 hours. Sodium acetate was added to the dialyzed suspension to 0.015 *M*, and the pH was adjusted to 6.8 with 0.1 *N* HCl. Chilled acetone was added to produce successive concentrations of 10, 20, 30, 40, and 50% acetone, and after each addition the resulting precipitate was recovered by centrifugation and taken up in 0.05 *M* phosphate buffer. To the supernatant remaining after each centrifugation was added the next increment of acetone. Each of the suspended precipitates was dialyzed against distilled water at 4° C., then dried in a current of warm (40-45° C.) air. The prepared fractions were suspended in Ringer-phosphate solution to a concentration of 0.5 mg. per milliliter and tested for spermicidal activity. The results of this trial, which are presented in Table 3, indicate that the fractions prepared with 10 and 20% acetone were toxic to spermatozoa, whereas the fractions prepared with 30, 40, and 50% acetone were relatively nontoxic.

Two lots of lactenin were prepared from separate lots of raw skimmilk by the acetone fractionation procedure. Inasmuch as the previous trial showed

TABLE 3

Effect of lactenin prepared by acetone fractionation and suspended in Ringer-phosphate solution on the mean per cent of motile spermatozoa in four ejaculates

	Hours of storage at 37° C.			
	1	2	4	6
	(%)	(%)	(%)	(%)
Ringer-phosphate	41	30	16	10
10% acetone fraction	15	7	0	0
20% acetone fraction	25	12	3	0
30% acetone fraction	40	28	15	10
40% acetone fraction	35	30	23	7
50% acetone fraction	40	30	15	10

little or no toxicity associated with the fractions prepared with the higher concentrations of acetone, only the 10 and 20% acetone fractions were prepared in these trials. The dried fractions were suspended at the rate of 0.5 mg. per milliliter of heated skimmilk, and separate tests of motility survival at 37° C. with six ejaculates were conducted on each lot of lactenin fractions. Since the results were similar in the two trials, the data were combined and are presented in Table 4.

TABLE 4

Effect of lactenin prepared by acetone fractionation of raw skimmilk on the mean per cent of motile spermatozoa in 12 ejaculates

	Hours of storage at 37° C.			
	1	2	4	6
	(%)	(%)	(%)	(%)
Heated skimmilk	55	51	43	33
10% acetone fraction	43	35	19	3
20% acetone fraction	39	27	13	7

Two additional lots of lactenin were prepared from lots of skimmilk of known low spermicidal activity: spermatozoa retained 50 to 60% of their initial motility after 4 hours of storage at 37° C. in the unheated skimmilks. Attempts were made to concentrate lactenin by the acetone fractionation procedure from these skimmilks, and no toxicity to spermatozoa was found when the dried fractions were employed at concentrations of 0.5-1.0 mg. per milliliter of heated skimmilk and stored at 37° C. These results indicate the difference in the initial toxicity of various lots of skimmilk.

Lactenin was prepared next from nonfat dry milk solids produced and marketed for human consumption. After reconstitution of the milk solids, the general procedure was the same as that outlined previously for raw skimmilk. In previous trials after the preliminary acetone treatment and recovery of the precipitate for dialysis, the supernatant was discarded. In this trial the supernatant was stored at 4° C., and after 5 days a white precipitate settled out. The precipitate was recovered, dried, and included in the livability trial as Fraction A.

After dialysis of the preliminary acetone precipitated material, acetate addition and pH adjustment, the preparation was centrifuged in the cold for 15 minutes, and the precipitate recovered in this manner is referred to as Fraction B. The supernatant from Fraction B was subjected to successive additions of chilled acetone to produce concentrations of 5, 10, 15, and 20% acetone, and the precipitate was recovered after each addition of acetone. Each of the six fractions was suspended in heated skimmilk at the rate of 0.5 mg. per milliliter and tested for spermicidal activity. The mean motilities for six ejaculates after storage at 37° C. are shown in Table 5. Although some toxicity was exhibited by each of the six fractions, Fraction A appeared to be relatively nontoxic, and most of the toxicity was concentrated in the 5 and 10% acetone-precipitated fractions.

TABLE 5

Effect of lactenin prepared by acetone fractionation of nonfat milk solids on the mean per cent of motile spermatozoa in six ejaculates

	Hours of storage at 37° C.			
	1	2	4	6
	(%)	(%)	(%)	(%)
Heated skimmilk	55	50	48	33
Fraction A	55	53	40	15
Fraction B	58	38	30	5
5% acetone fraction	48	20	0	0
10% acetone fraction	22	10	2	0
15% acetone fraction	52	48	30	7
20% acetone fraction	55	50	40	1

DISCUSSION

The results obtained with peroxidases seem to preclude any possibility that lactoperoxidase is an important spermicidal factor which is inactivated by the heating of milk. The peroxidases tested significantly increased the survival of spermatozoa stored at 4° C. in heated skimmilk, in agreement with the results obtained by VanDemark *et al.* with catalase *(16)*. Catalase and peroxidase exert similar effects in that both destroy hydrogen peroxide, which is produced by spermatozoa and is harmful to them *(15)*.

The trials with lactenin prepared by two different procedures clearly demonstrate its spermicidal activity and indicate that lactenin probably accounts for much of the spermicidal activity of unheated milk. Although the possibility of the existence of other factors can not be excluded, spermicidal lactenin fractions were recovered from all lots of milk which were spermicidal before fractionation. On the other hand, no activity was found in attempts to concentrate lactenin from skimmilk which was originally low in toxicity. The absence of lactenin activity from lots of skimmilk consisting of mixed milk from several herds is puzzling, although investigators *(6, 17)* have stated that the lactenin titer varies considerably with individual cows. Perhaps the most logical explanation is inactivation during processing in the plant. If lactenin functions through its influence on oxidation-reduction potentials, as suggested by Wilson and Rosenblum *(18)*, such inactivation could occur rather easily.

Attempts to purify lactenin have not been successful and its chemical nature has not been determined, although Jones and Simms *(8)* reported that no elements other than carbon, hydrogen, oxygen, and nitrogen were present. The problem of purification is complicated by the fact that lactenin is inactivated by many of the reagents ordinarily used for protein fractionation *(17)*. It apparently is not identical with any of the known antibacterial enzymes, including peroxidase and lysozyme, which are present in milk *(18)*. Although its properties, summarized by Wilson and Rosenblum *(17)*, have led investigators to believe that lactenin is an enzyme, there is no conclusive evidence that this is so. Acetone fractions, although higher in activity than preparations obtained by other methods, still were not pure when examined electrophoretically *(1)*.

In the trials reported in this paper, acetone-prepared fractions appeared to have more spermicidal activity than lactenin prepared by trypsin digestion and alcohol precipitation, in spite of the combined effects of trypsin and lactenin in the latter.

The toxicity of the fractions precipitated at lower concentrations of acetone and the absence of toxicity in those fractions precipitated at higher acetone concentrations indicates that the lactenin-2 fraction described by Auclair and Hirsch *(2)* is spermicidal, whereas the lactenin-1 fraction does not appear to affect spermatozoa. Inasmuch as lactenin-1 loses nearly all of its inhibitory activity after being heated to 70° C. for 20 minutes, it is possible that the heat treatment necessary to inactivate the rennin destroyed much of the lactenin-1 activity. The observation of Thacker *et al. (14)* that a small amount of unheated milk added to heated milk resulted in toxicity, might be advanced as evidence of a role of lactenin-1 in the toxicity for spermatozoa.

SUMMARY

Lactenin, an antistreptococcal substance of milk, has been concentrated by acetone fractionation of whey and shown to be highly toxic to bovine spermatozoa.

Lactenin prepared by tryptic digestion of whey, dialysis, and alcoholic precipitation also was toxic to spermatozoa, but the results were partially confounded by the difficulty of separating trypsin from the lactenin.

Lactoperoxidase, when added to heated skimmilk and used as a semen diluent in a storage trial, exhibited no toxicity for bovine spermatozoa at the concentration used.

REFERENCES

(1) Auclair, J. E., and Berridge, N. J. The Inhibition of Micro-organisms by Raw Milk. II. The Separation and Electrophoretic Examination of Two Different Inhibitory Fractions. *J. Dairy Research*, 20: 370. 1953.

(2) Auclair, J. E., and Hirsch, A. The Inhibition of Microorganisms by Raw Milk. I. The Occurrence of Inhibitory and Stimulatory Phenomena. Methods of Estimation. *J. Dairy Research*, 20: 45. 1953.

(3) Elliott, K. A. C. Oxidations Catalysed by Horse-radish- and Milk-peroxidases. *Biochem. J.*, 26: 1281. 1932.

(4) Green, N. M. Competition among Trypsin Inhibitors. *J. Biol. Chem.*, 205: 535. 1953.

(5) Johnson, P. E., Flipse, R. J., and Almquist, J. O. The Effect of Cysteine Hydrochloride on the Livability of Bull Spermatozoa in Unheated Skimmilk. *J. Dairy Sci.* (Abs.), 37: 650. 1954.

(6) Jones, F. S., and Simms, H. S. Adaptation of Mastitis Streptococci to Milk. *J. Exptl. Med.*, 50: 279. 1929.

(7) Jones, F. S., and Simms, H. S. The Bacterial Growth Inhibitor (Lactenin) of Milk. I. The Preparation in Concentrated Form. *J. Exptl. Med.*, 51: 327. 1930.

(8) Jones, F. S., and Simms, H. S. The Present Status of Lactenin. *Science*, 72: 456. 1930.

(9) McMeekin, T. L., and Polis, B. D. Milk Proteins. *Advances in Protein Chem.*, 5: 201. 1949.

(10) Polis, B. D., and Shmukler, H. W. Crystalline Lactoperoxidase. I. Isolation by Displacement Chromatography. *J. Biol. Chem.*, 201: 475. 1953.

(11) Singher, H. O. Personal Communication. 1953.

(12) SNEDECOR, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1946.

(13) THACKER, D. L., AND ALMQUIST, J. O. Diluters for Bovine Semen. I. Fertility and Motility of Bovine Spermatozoa in Boiled Milk. *J. Dairy Sci.*, **36**: 173. 1953.

(14) THACKER, D. L., FLIPSE, R. J., AND ALMQUIST, J. O. Diluters for Bovine Semen. II. Effect of Milk Proteins upon Spermatozoan Livability. *J. Dairy Sci.*, **37**: 220. 1954.

(15) TOSIC, J., AND WALTON, A. Formation of Hydrogen Peroxide by Spermatozoa and Its Inhibitory Effect on Respiration. *Nature*, **158**: 485. 1946.

(16) VANDEMARK, N. L., SALISBURY, G. W., AND BRATTON, R. W. Oxygen Damage to Bull Spermatozoa and Its Prevention by Catalase. *J. Dairy Sci.*, **32**: 353. 1949.

(17) WILSON, A. T., AND ROSENBLUM, H. The Antistreptococcal Property of Milk. I. Some Characteristics of the Activity of Lactenin in Vitro. The Effect of Lactenin on Hemolytic Streptococci of the Several Serological Groups. *J. Exptl. Med.*, **95**: 25. 1952.

(18) WILSON, A. T., AND ROSENBLUM, H. The Antistreptococcal Property of Milk. II. The Effects of Anaerobiosis, Reducing Agents, Thiamine, and other Chemicals on Lactenin Action. *J. Exptl. Med.*, **95**: 39. 1952.

THE EFFECT UPON MILK PRODUCTION AND BODY WEIGHT OF VARYING WITHDRAWAL PERIODS AFTER THYROACTIVE SUPPLEMENT FEEDING

E. W. SWANSON

Dairy Department, University of Tennessee, Knoxville

The ability of thyroactive supplemental feeding to cause increases in milk production of dairy cows in the declining phase of lactation has been demonstrated by several independent investigators, most of whose results have been reviewed by Blaxter *et al. (3, 4)*. Observed production increases have been accompanied by moderate to large losses in body weight in spite of extra feed, followed by a return to normal rate of gain as the production stimulus declined *(10, 13, 14)*. It also has been noted that after abrupt withdrawal of the thyroactive supplement, milk production has declined very rapidly to a subnormal level, and in some cases lactation has virtually ceased *(2, 5, 8)*. For this reason the common method of using thyroactive supplement has been to continue feeding it until time to dry off the cow. Since the period of significant stimulus has been about 10 to 14 weeks in most experiments *(7, 10)*, it would be economically advantageous to withdraw the supplement from the ration at that time, or earlier if desired, if such withdrawal could be done without causing a decline below normally expected production. Gradual withdrawal has been proposed to produce this effect *(2)*. Another question regarding the economy of thyroactive supplement feeding concerns the nature of the body weight losses and gains. It has been postulated that the weight changes may be due to water balance shifts *(13)*. The improbability of the rapid weight gains, 4 to 5 lb. daily, after withdrawal of the thyroactive feed being comparable to normal weight gains has been emphasized in the light of insignificant changes in nutrient intake *(6, 13)* or digestion efficiency *(1)*. If the lost weight is regained without significant increases in nutrient intake, the energy represented by such weight losses cannot be charged properly against the treatment period. This study was conducted to determine the gradual withdrawal period following thyroactive feeding which would produce the least loss of production, if any, below normal and to identify the nature of the primary weight losses and gains associated with thyroactive feeding.

EXPERIMENTAL METHODS AND RESULTS

Experiment I

A limited study of different withdrawal rates was made after a 100-day period of feeding Protamone[1] to eight cows which were compared with eight cows not fed the thyroactive material *(11)*. The eight treated cows were divided

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[1] Protamone brand of iodinated casein was furnished for this experiment through the courtesy of Dr. George O. Kohler, Cerophyl Laboratories, Kansas City, Missouri.

into three groups to give comparable rates of production decline among the groups. Beginning at the 101st day the 3 lb. of supplemental feed containing 15 g. of Protamone was gradually withdrawn from the groups in 10, 15, and 30 days, respectively. Daily milk weights were recorded and weekly milk fat tests were made.

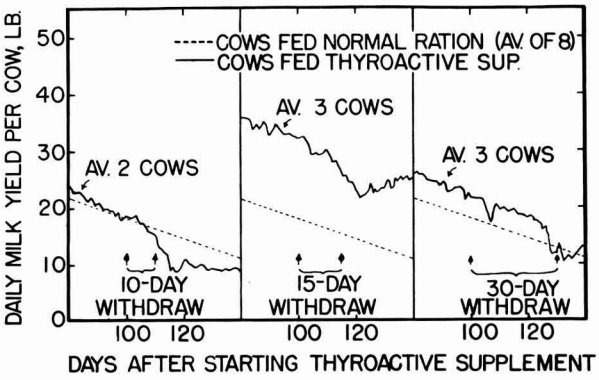

FIG. 1. Average milk production changes of cows fed Protamone followed by 10-, 15-, and 30-day withdrawal periods.

The results of this comparison are presented in Figure 1. These data indicate that the treated cows at this time were declining at about the same rate as the control cows. During a 10-day withdrawal period daily milk yields declined rapidly and continued to decline for 6 more days, after which they leveled off. The break in rate of decline in the 15-day withdrawal came about 5 days later than that in the 10-day group, but still continued for 5 days after withdrawal and then leveled off. The group withdrawn over 30 days followed the normal decline rate until the 27th day, after which a sharp decline occurred during the next 6 days, followed by a leveling off. These results indicated that 10- and 15-day periods were too short to prevent a marked decline of 7 to 8 lb. of milk daily when the thyroactive feed was withdrawn but that the decline was quite uniform with a terminal drop of only about 4 lb. of milk daily after a 30-day withdrawal period.

Experiment II

Because of the small number of cows in each group in the preliminary withdrawal comparisons, another comparison was made with six carefully paired cows in each group. Three pairs were Jerseys and three pairs were Holstein-Friesians. One of the six pairs was a set of identical twin heifers, which were unusually well paired even for identical twins. After a 30-day preliminary period, 3 lb. of thyroactive feed supplement containing 15 g. of Protamone were fed to each cow daily for 70 days. Withdrawal of the supplement was made gradually in 25 days in one group and in 18 days in the second group. It was

hoped that these periods might show intermediate effects between those of the previous 15- and 30-day periods. All cows were several weeks past the peak of lactation, and the groups averaged 17 weeks in lactation when treatment started. All milk weights were recorded and daily composite samples were taken for fat test once a week. Body weight, heart girth, and paunch girth were taken on the same day for all cows at weekly intervals. A comparable group of six control cows was used for body weight and measurement comparisons, but not for production comparisons. Rates of grain feeding were equal between groups. All cows were fed mixed hay and corn silage ad lib. along with rye pasture when available at infrequent intervals.

Production changes. The weekly average milk production of the two withdrawal groups is shown in Figure 2. The rate of decline was very high for both groups throughout the experiment. This may have been conditioned by the hot weather and severe fall drought at the start of this experiment. The groups

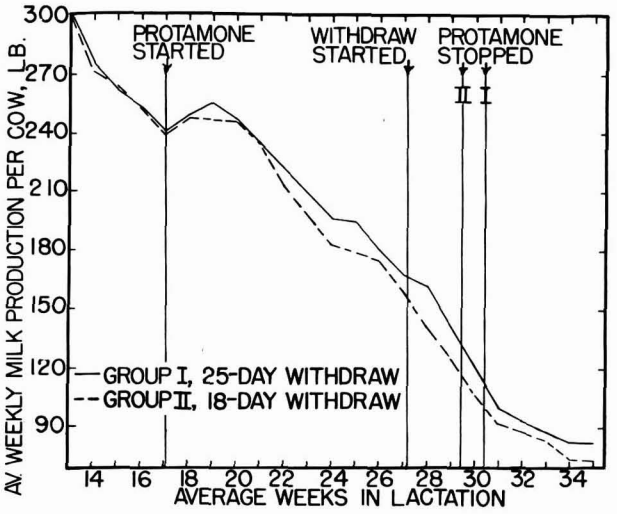

FIG. 2. Milk production changes of two groups of six cows each comparing 18- and 25-day withdrawal after feeding Protamone for 70 days.

responded comparably to the thyroactive supplement. Two cows in each group developed clinical mastitis during the 70-day treatment period. They were not eliminated because the effects were about the same on each group average, but these attacks did result in a higher rate of decline than normal during the last part of the experiment. The rates of decline during the withdrawal periods were nearly the same, and both groups leveled off at above their normal expected production based upon an extension of their pretreatment rate of decline.

The production data from the pair of identical twins and an untreated control heifer comparable to the twins are plotted in Figure 3. No health or management problems occurred in these animals except the failure of Twin 6 to eat

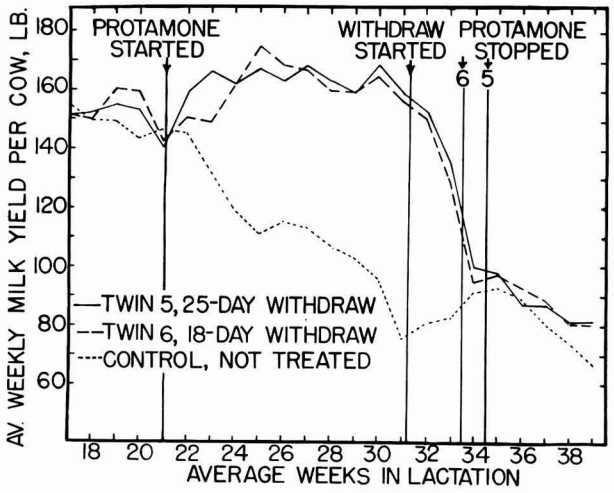

FIG. 3. Milk production changes of a nontreated control heifer and a pair of identical twins during Protamone feeding and 18- and 25-day withdrawal periods.

all of her supplement during the first 10 days. After that, production of the twins was nearly identical each week during the treatment period, during the variable withdrawal periods, and during the 30-day post-treatment period. The production response of these heifers to treatment was marked, and it remained high until withdrawal. The post-treatment production, although it was about 7 lb. per day below the prewithdrawal yields, was not different from the expected normal production based upon either the control heifer or an extension of the pretreatment decline rate.

Body measurement changes. The weekly average body weights and paunch girth measurements for the two treated groups and a nontreated control group are plotted in Figure 4. Heart girth measurements followed the same pattern but to a lesser degree of change. The control group gradually increased in body weight and paunch girth throughout the observation period. After Protamone feeding was started, the two treated groups lost weight and paunch girth consistently for 6 weeks. The average weight losses were 70 to 75 lb. and the girth reductions were 3.5 to 4.5 in. A slight increase in weight and girth then began, but after 5 more weeks when withdrawal of the supplement had been started, the treated cows still averaged 54 lb. below their pretreatment weight and 3.5 in. below their pretreatment girth. At the same time the nontreated cows had gained 39 lb. and 0.8 in.

As shown in Figure 4, there were no significant differences between the treated groups in the rate of body measurement changes during the variable withdrawal periods. The large variations at the 29th and 30th week were due to widely varying weather conditions. Rapid gains in body weight started during the second week of withdrawal in both treated groups and continued for 3 to 4

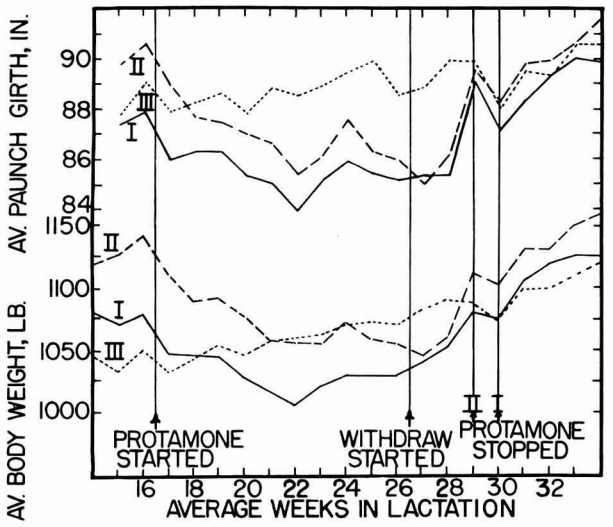

FIG. 4. Body weight and paunch girth changes of two groups of cows, I and II, fed Protamone compared with a control group, III.

weeks after treatment had stopped. By this time the treated cows had gained 90 to 100 lb., compared to 40 lb. for the untreated group, and all groups were again in the same relative weight positions as in the pretreatment period. Increases in paunch girth in the treated groups averaged 5.5 in., compared to only 0.7 in. for the controls. This restored the relative paunch girth positions of the three groups which existed in the pretreatment period.

Experiment III

Body water changes. The possibility of large shifts in the water content of the cows occurring during and after thyro-stimulation was investigated by determining body water by the antipyrine dilution method *(9)*. In order to relate body water to the normal body weights observed, the animals were not fasted before being dosed intravenously with 10 to 15 g. of antipyrine. After injection, the heifers were tied so they could not obtain water or feed until after the sampling period, 4.5 to 5.0 hours. Two sets of identical twins were used in which one of each pair was treated and the mate was the control. One of the treated twins metabolized the antipyrine so rapidly that unreliably low blood values were obtained; therefore, data are presented for only one of these pairs, No. 18 and 19. The two twins which were both treated in Experiment II were also used for body water determinations and these were compared with a comparable control heifer.

The estimations of per cent body water derived by the antipyrine dilution method are presented in Table 1. Rather wide variations were observed between heifers and in the same heifer at different times. The general trend in both

TABLE 1

Total body water content of normal and Protamone-fed cows during and after the treatment periods

Period	Date	Stage of expt.	Protamone-fed		Controls	
			Cow No.	Body water	Cow No.	Body water
		(Days)		*(%)*		*(%)*
During treatment	7/28	45	19	69	18	62
	10/8	117	19	59	18	72
	12/18	69	5	80	3	79
		69	6	69		
Average				69		71
After withdrawal	11/5	26	19	66	18	62
	12/3	54	19	56	18	59
	1/20	8	5	67	3	67
		15	6	68		
Average				64		63

control and treated heifers was toward lower body water percentages as the lactation advanced. The differences between control and treated heifers either during or after treatment are not significant. The data definitely fail to support the hypothesis that thyroactive supplement feeding at the levels used results in total body dehydration or that part of the rapid weight gain after treatment is due to restoration of normal water content. The supplement was stopped in 25 days for No. 5, in 18 days for No. 6, and abruptly for No. 19. These differences in withdrawal rate did not cause notable differences in body water in comparison with the controls.

DISCUSSION

The milk production changes during varying rates of withdrawal of thyroactive feed supplement indicated that yields could be prevented from dropping below the normal expected level if the withdrawal period exceeded 15 days. The most gradual decline occurred when the supplement was reduced at the rate of 0.1 lb. daily for 30 days. Differences between 15-, 18-, and 25-day periods were not noticeable. The results observed with individual cows indicated that the higher the stimulated production was above the expected normal yield, the more precipitous was the decline after withdrawal of the supplement. This is shown by comparing the lactation curves in Figures 2 and 3. All cows observed declined to their approximate normal yield after withdrawal, and it is highly improbable that a normal rate of decline can be obtained from a stimulated high level even by very gradual withdrawal. When the thyro-stimulation is removed, it must be expected that production will adjust to the normal level based upon stage of lactation and gestation, feed conditions, and other management factors affecting production. If this adjustment is 6 lb. per cow daily and the existing rate of decline is 0.1 lb. per day, the extension of that decline rate for 20 days will still require a drop of about 4 lb. to occur near the end of the withdrawal period. If the withdrawal period is shortened the terminal drop in yield will be greater, and if it is lengthened the drop will be less.

One of the theoretical advantages of a gradual over an abrupt withdrawal is the slow adjustment of the body to removal of exogenous thyroxine. During thyroactive supplement feeding the thyroid becomes inactive *(10, 12)*. How quickly it can resume normal operation after withdrawal has not been determined definitely. The large drops in yield after abrupt or short withdrawal periods indicate that thyroid normality is not achieved immediately. Activity of the thyroid as measured by uptake of radioactive iodine indicates that normal function is achieved by 4 weeks after abrupt withdrawal *(12)*, and the minimum time is probably less than this. In view of this fact and the insignificant difference between 18- and 25-day withdrawal periods in this study, 3-week withdrawal periods, or slightly less, should be satisfactory and gradual enough to prevent below-normal production dips in practical use.

This investigation has shown that the major element in the body weight changes during and after thyroactive supplement feeding is the variation in gastro-intestinal fill. The loss of 3.5 in. in paunch girth during the early weeks of thyroactive feeding would represent about 7.5 gal. capacity, or more than 60 lb. of weight, compared to 54 lb. average actual loss. The 5.5 in. increase in girth after withdrawal could represent about 89 lb. of weight, whereas the actual average increase of all of the treated cows at that time was 95 lb. The very minor changes in body water percentages at the same time indicate that very little, if any, of the weight change is due to unusual shifts in body water. A more logical explanation is that the thyroactive feed causes stimulation of the gastrointestinal tract, resulting in smaller capacity. Removal of the stimulation allows the organs to resume their normal condition. In view of this possibility, most of the body weight loss during thyroactive feeding cannot be considered as energy loss, and the weight gain after treatment cannot be considered energy gain. In order to make proper comparisons of energy balances on the basis of body weights, pretreatment weights should be compared with weights of 3 to 4 weeks after treatment in both treated and control cows.

SUMMARY

Withdrawal of 3 lb. of thyroactive feed supplement containing 15 g. Protamone daily from the ration of cows so fed for 10 to 14 weeks has been compared over periods of 10, 15, 18, 25, and 30 days. Withdrawal in 10 days was accompanied by a sharp drop in milk yield and a temporary period of subnormal production. Less rapid decline was noted in the 15-, 18-, or 25-day withdrawal periods, with little difference in yield decline rates noted between the last two periods. Yields did not drop below estimated normal production after withdrawal periods of 15 days or more. Extending the withdrawal period to 30 days did not prevent a terminal drop from a partially stimulated yield to the estimated normal level. Rapid gains in body weight occurred after thyroactive feeding ceased. These were accompanied by increases in paunch girth large enough to account for nearly all of the weight increase as gastrointestinal fill. The total body water contents during and after treatment were essentially the same in

control and treated groups, indicating very minor changes in water balance due to thyroactive feeding.

REFERENCES

(1) Balch, C. C., Balch, D. A., Bartlett, S., Johnson, V. W., and Rowland, S. J. Factors Affecting the Utilization of Food by Dairy Cows. *Brit. J. Nutrition*, 6: 356. 1952.

(2) Blaxter, K. L. Experiments with Iodinated Casein on Farms in England and Wales. *J. Agr. Sci.*, 36: 117. 1946.

(3) Blaxter, K. L. Some Effects of Thyroxine and Iodinated Casein on Dairy Cows, and Their Practical Significance. *Vitamins and Hormones*, 10: 217. 1952. Academic Press, New York.

(4) Blaxter, K. L., Reineke, E. P., Crampton, E. W., and Petersen, W. E. The Role of Thyroidal Materials and of Synthetic Goitrogens in Animal Production and an Appraisal of Their Practical Use. *J. Animal Sci.*, 8: 307. 1949.

(5) Booth, A. N., Elvehjem, C. A., and Hart, E. B. Some Effects of Feeding Iodinated Casein to Dairy Cows. *J. Dairy Sci.*, 30: 443. 1947.

(6) Gardner, K. E., and Millen, T. W. Thyroprotein for Lactating Cows in Midsummer. *J. Dairy Sci.*, 33: 531. 1950.

(7) Leech, F. B. The Galactopoietic Effect of Iodinated Casein: Dose Response Relationships During Prolonged Treatment. *Endocrinology*, 7: 42. 1950.

(8) Reece, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over an Extended Period. *J. Dairy Sci.*, 30: 313. 1947.

(9) Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hollander, V., and Steele, J. M. The Use of Antipyrine in the Measurement of Total Body Water in Man. *J. Biol. Chem.*, 179: 31. 1949.

(10) Swanson, E. W. The Effect of Feeding Thyroprotein to Dairy Cows during the Decline of Lactation in Successive Lactations. *J. Dairy Sci.*, 34: 1014. 1951.

(11) Swanson, E. W., and Hinton, S. A. The Economy of Winter Feeding Thyroactive Supplement under a Base-Surplus Marketing Plan. *J. Dairy Sci.*, 36: 582. 1953.

(12) Swanson, E. W., Monroe, R. A., and Comar, C. L. Using Identical Twin Dairy Cows to Determine the Effect of Iodinated Casein (Protamone) on Milk Production, Thyroid Activity, and Body Weight Changes. *J. Dairy Sci.*, 37: 659. 1954.

(13) Thomas, J. W., and Moore, L. A. Thyroprotein Feeding to Dairy Cows during Successive Lactations. *J. Dairy Sci.*, 36: 657. 1953.

(14) Thomas, J. W., Moore, L. A., and Sykes, J. F. Some Effects of Feeding Thyroprotein to Dairy Cows during Their First Lactation. *J. Dairy Sci.*, 32: 278. 1949.

THE ULNAR EPIPHYSEAL CARTILAGE WIDTH IN NORMAL AND RACHITIC CALVES AND ITS USE COMPARED TO OTHER METHODS OF DETECTING RICKETS

J. W. THOMAS, M. OKAMOTO, AND L. A. MOORE
Dairy Husbandry Research Branch
Agricultural Research Service, USDA, Beltsville, Maryland

The use of X-ray photographs of the ulnar epiphyseal cartilage to demonrate the presence of rickets in calves was first mentioned in 1938 by investitors from Pennsylvania State College *(1)*. More recently this technique has en used by the present investigators *(4, 5, 6)*. However, to date it has been ed only to differentiate between normal and rachitic calves.

It appeared desirable to make a detailed study of the normal pattern of sification of the epiphyseal cartilage of the ulna in calves similar to studies ade on the ossification centers in human wrists and hands *(2)*. A normal ttern for calves would thus be obtained. This would make it possible for yone to compare any roentgenogram with that of a normal which had been tablished for that particular breed, sex, and age. This would be used as an aid a person desiring to use this technique in the field for the detection of rickets. would also establish a physiological or skeletal age pattern based on one phase bone development.

The purpose of this study was to determine the normal pattern for the ossifition of the ulnar epiphyseal cartilage in young dairy calves and to present data which this method was compared with other commonly used methods for the tection of rickets.

PROCEDURE

X-ray photographs were obtained on 5 × 7 in. X-ray safety film. The film as placed in cassettes which were held with clamps and ringstand behind the stal end of the left ulna on the inner part of the leg at about a 50 to 60 degree gle from the anterior view with the calf in standing position. The X-ray tube as approximately 30 in. from the cassette at about a 60 to 75 degree angle with e anterior view. A photograph of this procedure is shown in Figure 1. A odel F portable General Electric X-ray unit was used and exposure at 15 mv. ried from ½ to 1 second, depending on the size of the calf. The procedure as similar to that mentioned by Bechdel *et al. (1)* except that it was more satisctory to hold the calves in a corner of the barn than to try to restrain them in crate. X-ray photographs of small calves were obtained from the horizontal sition with the left foreleg held above the cassette, which was on the floor, and e X-ray head above the leg at the appropriate angle and distance.

To represent normal development in calves, X-ray photographs were taken

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FIG. 1. Position of apparatus for taking X-ray photograph.

every 3 to 6 weeks during two alternate years on calves in the breeding herd and some normally-fed calves in the nutrition herd at Beltsville.

The width of the epiphyseal cartilage was determined by measuring its width in millimeters with calipers or dividers at five equally spaced intervals across its diameter on the X-ray negative. The five values were averaged and used as the width of the ulnar epiphyseal cartilage for the calf at that particular age. Values smaller than 1.0 mm. were difficult to measure accurately, and the use of this measurement on animals over 8 months of age was impractical. A sketch of the parts of the leg in a typical X-ray photograph is shown in Figure 2.

Regression coefficients were calculated between days of age and average cartilage width by means of the logarithm of average cartilage width at 10-day intervals of age from 0 to 240 days of age *(3)*.

Data on plasma calcium, serum phosphatase, and body weight gains, together with data on cartilage width, of calves fed dried beet pulp and grain with and without vitamin D are presented for comparative purposes. The care and procedure used in raising these calves and some of the data have been described previously *(4, 5, 6)*.

RESULTS AND DISCUSSION

The average ulnar epiphyseal cartilage width and its standard error for the two breeds and two sexes at monthly intervals are presented in Table 1. Data for the first month were divided into two intervals because of a more rapid ossification during this period. The number of calves observed is also given.

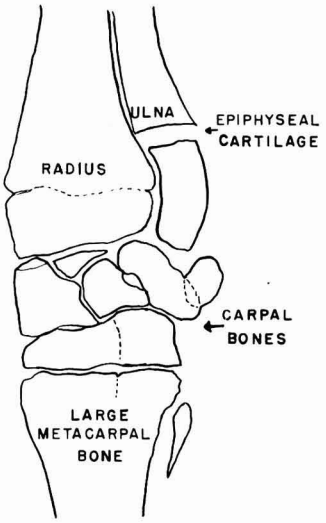

FIG. 2. A sketch of a typical X-ray photograph showing position of epiphyseal cartilage and other bones.

In general, the epiphysis was wider in young male calves than in female calves. The significance of these differences at the various ages is indicated toward the bottom of Table 1. Male calves usually showed a faster rate of ossification than did female calves. A similar sex difference has been demonstrated in humans *(2)*.

The cartilage width of Holstein calves was greater than that of Jersey calves for the first 2 to 3 months of age. After this time the breed difference usually was not significant.

A straight line was formed when logarithm of ulnar epiphyseal cartilage width was plotted against age of the calves. The formulas for calculating expected values for calves of both breeds and sexes are given at the bottom of Table 1, together with the standard error of estimates for Y. The regression coefficients were not significantly different when a covariance analysis *(3)* was applied to them, although the difference between the sexes approached significance. The correlation coefficients for Holstein females, Holstein males, Jersey females, and Jersey males were 0.99, 0.97, 0.98, and 0.98, respectively.

The ulnar epiphyseal cartilage width of calves of the same sex and breed that were born in winter months was the same as that of calves born in the summer months and both were the same as calves born in the spring and autumn months.

A comparison of the usual blood constituents employed in the detection or study of rickets and the cartilage width in normal and rachitic calves is shown in Table 2. The significance of the differences between the two groups of calves at the various ages also is shown.

TABLE 1

Observed ulna epiphyseal cartilage width of normal calves at various ages with significance of differences found and formulas for expected values

	Age in days								
	0-10	11-30	31-60	61-90	91-120	121-150	151-180	181-210	211-240
Holstein females									
Number	27	26	46	35	37	35	34	29	25
Av. *(mm.)*	7.69	6.49	5.04	4.04	3.01	2.33	1.61	1.10	0.92
St. error	±.20	±.18	±.17	±.13	±.13	±.11	±.11	±.08	±.08
Holstein males									
Number	30	26	35	33	28	24	18	11	9
Av. *(mm.)*	8.71	7.56	5.95	4.40	3.85	2.63	2.42	1.42	1.23
St. error	±.27	±.31	±.20	±.14	±.18	±.36	±.15	±.09	±.25
Jersey female									
Number	15	14	20	19	17	12	16	15	16
Av. *(mm.)*	6.90	5.69	4.21	3.20	2.74	2.18	1.39	1.19	0.65
St. error	±.14	±.19	±.16	±.16	±.12	±.11	±.11	±.10	±.08
Jersey male									
Number	15	20	21	24	19	25	15	12	24
Av. *(mm.)*	7.39	6.31	5.19	4.28	3.60	2.79	1.88	1.39	.80
St. error	±.32	±.26	±.19	±.21	±.19	±.13	±.16	±.19	±.11
Significances of differences, P =									
Sex difference									
Holsteins	<0.01	<0.01	<0.01	—	<0.01	—	<0.01	0.05	—
Jerseys	—	—	<0.01	<0.01	<0.01	0.01	0.02	—	—
Breed difference									
Between males	<0.01	<0.01	0.05	—	—	—	0.02	—	—
Between females	<0.01	<0.01	<0.01	<0.01	—	—	—	—	0.05

H ♀ $\hat{Y} = 1.91444 - 0.0042924\ X\ (\pm 0.04991)$

H ♂ $\hat{Y} = 1.95246 - 0.0038408\ X\ (\pm 0.06611)$

J ♀ $\hat{Y} = 1.84761 - 0.0042798\ X\ (\pm 0.06375)$

J ♂ $\hat{Y} = 1.92524 - 0.0040610\ X\ (\pm 0.06348)$

All ♀ $\hat{Y} = 1.88103 - 0.0042861\ X\ (\pm 0.05599)$

All ♂ $\hat{Y} = 1.93885 - 0.0039509\ X\ (\pm 0.06386)$

All calves $\hat{Y} = 1.90994 - 0.0041185\ X\ (\pm 0.06189)$

$\hat{Y}$ = Log. of width in mm. × 10

X = days of age

TABLE 2

Cartilage width, blood values, and growth of calves receiving dried beet pulp and grain with or without vitamin D

Vit. D	Item	Age in days							
		10-30	31-60	61-90	91-120	121-150	151-180	181-210	211-240
+	Ulna epiphyseal cartilage width *(mm.)*	6.57 ±0.62	5.26 ±0.38	4.19 ±0.26	3.38 ±0.13	2.69 ±0.20	2.00 ±0.20	1.04 ±0.19	0.38 ±0.24
−		6.33 ±0.66	5.73 ±0.26	6.14 ±0.34	6.79 ±0.71	6.75 ±0.59	5.95 ±0.86	—	—
Significance of difference, P =		—	—	<0.01	<0.01	<0.01	<0.01	—	—
+	Plasma calcium *(mg. %)*	10.18 ±0.28	10.66 ±0.16	11.11 ±0.27	10.95 ±0.23	10.81 ±0.33	10.90 ±0.23	11.29 ±0.06	11.21 ±0.43
−		10.51 ±0.98	9.59 ±0.31	8.15 ±0.32	7.59 ±0.46	7.55 ±0.35	7.91 ±1.24	—	—
Significance of difference, P =		—	0.02	<0.01	<0.01	<0.01	<0.01	—	—
+	Serum phosphatase *(Bodansky units)*	6.39 ±0.78	8.24 ±0.57	9.98 ±0.82	8.53 ±0.90	8.77 ±0.98	8.10 ±1.06	8.86 ±0.37	8.12 ±0.52
−		7.00 ±6.81	11.04 ±1.08	17.95 ±1.05	18.01 ±1.53	18.96 ±1.70	18.97 ±3.98	—	—
Significance of difference, P =		—	0.05	<0.01	<0.01	<0.01	<0.01	—	—
+	Body weight gain *(% of normal)*	67.8 ±2.4	99.0 ±5.3	92.3 ±5.0	71.2 ±6.1	76.4 ±4.2	97.6 ±4.9	87.0 ±8.0	97.3 ±7.5
−		73.3 ±2.3	90.9 ±6.4	73.5 ±4.6	32.2 ±5.5	32.8 ±8.9	18.0 —	—	—
Significance of difference, P =		—	—	0.02	<0.01	<0.01	<0.01	—	—
+	No. of calves	10	10	10	10	10	6	5	4
−		14	14	13	13	12	3	0	0

During the second month of life both blood calcium and phosphatase values indicated a difference between these two groups of calves at $P = 0.02$ and $P = 0.05$, respectively. After 60 days of age a measure of cartilage width, plasma calcium, serum phosphatase, and relative rate of body weight gain all showed significant differences between these two groups of calves. With proper use and interpretation, our data indicate that values for calcium, phosphatase, and cartilage width have approximately equal ability to permit detection of rickets in young calves.

An expression which would be of more definite help in the detection of rickets would be to observe two values at different times and express these values as a rate of change per unit time. For instance, the rate of ossification of the ulnar epiphyseal cartilage in microns per day for calves 2 and 3 months of age showed a significant difference [$P < 0.01$ between the values for these two groups ($+35$ and -17 μ/day)]. Similar comparisons for blood calcium and phosphatase showed significant differences ($P < 0.01$) in rates of change for the two groups of calves.

All these methods can be used to follow the rate of healing in rachitic animals. For example, a 4-month-old calf fed grain and whole milk became rachitic in late December. Analysis showed that plasma calcium was 7.7 mg.%; serum phosphatase was 20.4 Bodansky units, and cartilage width was 9.9 mm. The calf was given 10,000 I.U. of vitamin D per day plus some bone meal and was allowed access to sunshine. A month later the respective values were 10.5, 11.0, and 4.0.

The X-ray method of detecting rickets has certain advantages over the chemical methods that require access to a laboratory. It gives a permanent physical record that is not subject to error of determination. The general state of bone calcification also can be estimated from the roentgenogram.

In contrast to blood data, where the values for the rachitic animal deviate from a stationary normal value, in the cartilage width method the normal value changes according to a definite pattern, whereas the cartilage width of rachitic animals remains more or less stationary.

The establishment of normal cartilage development at various ages may prove useful also in certain physiological or skeletal age studies. The cartilage area of the ulna of calves is apparently the most satisfactory area for observation and measurement, since other cartilage areas are not wide and become ossified at an earlier age. In developing a physiological or skeletal age pattern in humans the use of ossification centers has been given more emphasis than the ankylosis of cartilage areas of the bones *(2)*. In calves these ossification centers occur prepartum and cannot be used for this purpose. It was noticed that the carpal bones, especially the accessory carpal, develop and enlarge during the first few months of life. Their development could be used to determine a physiological or skeletal age pattern, but it does not offer as satisfactory a method of measurement as does the ulnar epiphyseal cartilage width.

SUMMARY

Observed values have been presented for the width of the epiphyseal cartilage of the ulna for Holstein and Jersey calves up to 240 days of age. It was wide in young calves and gradually became ossified or "closed" at about 8 months of age. Formulas were derived and presented from which expected values can be obtained at any given age.

A significant difference in the cartilage width between breeds and sexes was found at certain ages. There was no seasonal trend or differences in these values for calves born in winter, summer, or spring and fall.

Examples are given of the use of this technique in the detection of rickets, in which values for blood calcium and phosphatase were compared to cartilage width in normal and rachitic calves.

The use of this technique to determine physiological or skeletal age in young calves is indicated.

REFERENCES

(1) Bechdel, S. I., Hilston, N. W., Guerrant, N. B., and Dutcher, R. A. The Vitamin D Requirement of Dairy Calves. Pa. Agr. Expt. Sta., *Bull.* 364. 1938.

(2) Greulick, W. W., and Pyle, S. I. *Radiographic Atlas of Skeletal Development of the Hand and Wrist.* Stanford University Press, Stanford, Calif. 1950.

(3) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(4) Thomas, J. W. The Use of X-Ray Photographs in the Detection of Rickets in Calves. *BDIM-Inf*-95. June, 1950.

(5) Thomas, J. W. Antirachitic Activity of Some Dehydrated Alfalfa Hays and the Effect of Adding Limestone to the Grain Ration on the Development of Rickets in Calves. *J. Dairy Sci.*, **35**: 1107. 1952.

(6) Thomas, J. W., and Moore, L. A. Factors Affecting the Antirachitic Activity of Alfalfa and Its Ability to Prevent Rickets in Young Calves. *J. Dairy Sci.*, **34**: 916. 1951.

THE THYROXINE CONTENT OF THYROACTIVE IODINATED PROTEINS AS DETERMINED BY A RADIOACTIVE ISOTOPE DILUTION TECHNIQUE[1]

E. P. REINEKE

Department of Physiology and Pharmacology
Michigan State College, East Lansing

During recent years the effects produced by thyroactive iodinated proteins on milk secretion and associated processes have been investigated extensively, as indicated in the reviews by Blaxter *et al. (1)* and Sykes *et al. (14)*. The physiological effects of such preparations have been attributed to their thyroxine content, and in fact thyroxine can be isolated from them readily subsequent to hydrolysis. However, there has been considerable question regarding the actual amount of thyroxine present. Bio-assays of iodinated proteins administered parenterally to guinea pigs yielded values that agreed within 10% with their apparent thyroxine content *(13)*, as determined by a butanol extraction technique. However, a much greater response to iodinated proteins than to comparable doses of thyroxine is obtained in frog tadpoles *(11)*, and a smaller response is obtained in assays by the goiter prevention method in rats *(5)* Because of these species idiosyncrasies there has been considerable question as to how closely the results of a biological assay of an iodinated protein will represent its true thyroxine content. In an earlier report *(12)* it was pointed out that the actual yield of thyroxine obtained by isolation is far below the values indicated by biological tests. More recently, it has been found by paper chromatography *(3, 7)* that hydrolyzates of iodinated proteins contain at least two iodinated butanol-soluble compounds (diiodothyronine and triiodothyronine) in addition to thyroxine. These compounds would be included with the thyroxine fraction in the n-butanol extraction technique for thyroxine analysis.

In view of these considerations it was decided to employ a radioactive isotope dilution technique in an attempt to determine the true thyroxine content of thyroactive iodinated proteins.

EXPERIMENTAL PROCEDURE

Seven different lots of Protamone, a thyroactive iodinated casein containing n-butanol soluble iodine equivalent to approximately 3.0% thyroxine, were employed in the series of analyses.

Preparation of radioactive thyroxine. In the initial trials, radioactive thyroxine was prepared by iodination of casein *(11)*. Forty g. of casein were suspended in 1,400 ml. of distilled water, containing 10.0 g. of $NaHCO_3$ at 40° C. Approximately 2.5 mc. NaI^{131} solution was placed in a test tube, and the iodine

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was liberated by adding a crystal of KI plus an excess of KIO_3, followed by three drops of glacial acetic acid. Concentrated NH_4OH solution was then added until the iodine color disappeared. The radioactive solution was added to the previously prepared casein solution. The iodination was completed by adding, in small portions and with continual stirring, 7.4 g. of powdered I_2. The temperature was then increased to 70° C., and the solution was maintained at this temperature, with constant stirring, for 20 hours. After recovery of the iodinated casein, radioactive thyroxine was isolated and purified as described by Reineke and Turner *(12)*. In later work, radioactive thyroxine was formed by iodination of 3,5-diiodothyronine. The I^{131} was released as already described and the remainder of the process conducted by the procedure of Borrows *et al. (2)*. Purification was effected by first washing the crude thyroxine with several changes of absolute ethyl alcohol containing a few drops of glacial acetic acid and then recrystallizing alternately as the monosodium salt and the free compound as described previously. After drying to constant weight in a desiccator at room temperature, a stock solution was prepared by dissolving a weighed quantity of radioactive thyroxine in an accurately determined volume of 70% alkaline alcohol, usually in a concentration of 1.0 mg. per milliliter.

Stability of I^{131}-labeled thyroxine during hydrolysis. The analytical procedure devised depends on the extent of dilution of added radioactive thyroxine by the nonlabeled thyroxine isolated from hydrolyzates of iodinated protein.

Inasmuch as it has been shown that under certain conditions iodine will exchange readily between iodide and thyroxine *(4)*, it seemed essential to determine whether exchange would occur during the conditions of hydrolysis to be employed.

One ml. of stock solution, containing 5.4 mg. of radiothyroxine, and 100 mg. of nonlabeled thyroxine were placed in a small boiling flask. To this were added 21 ml. of distilled water, 10.6 g. $Ba(OH)_2 \cdot 5H_2O$ and 34 g. of KI. The mixture was refluxed for 20 hours in a boiling water bath. After cooling, the barium hydroxide was neutralized with dilute HCl and adjusted to pH 5.0. The precipitate was recovered by centrifuging, dissolved in 25 ml. of $N/_{10}$ NaOH solution, heated to 90° C., and treated with 5.0 ml. of saturated Na_2SO_4. Further purification was effected by dissolving the precipitate in 2 ml. of boiling sodium carbonate solution and chilling it to crystallize the thyroxine as the monosodium salt. Finally, the thyroxine was dissolved in 70% alkaline alcohol and crystallized from the boiling solution by adding glacial acetic acid. The crystals were collected in a sintered filter and dried to constant weight. Radioactivity counts were made on weighed samples of the recovered thyroxine and compared with aliquots of the original radioactive stock solution.

The results are summarized in Table 1. The actual recovery of thyroxine in the two samples was 78.2 and 79.7%. However, the radioactivity of the recovered thyroxine was 98.1 and 100.3% of what would be expected from the amount of labeled thyroxine included initially. From these results it is concluded that under the conditions of hydrolysis employed no significant exchange of iodine between thyroxine and iodide occurs.

TABLE 1

Recovery of labeled thyroxine after refluxing in barium hydroxide solution

	Sample 1	Sample 2
Radioactive thyroxine added *(mg.)*	5.4	5.4
Total thyroxine added *(mg.)*	105.4	105.4
Thyroxine recovered *(mg.)*	82.4	84.0
Per cent recovery	78.2	79.7
Radioactive thyroxine counted *(mg.)*	0.54	0.54
Counts per minute	2,145	2,145
Recovered thyroxine counted *(mg.)*	11.0	11.0
Counts per minute	2,195	2,245
Expected count	2,238	2,238
Per cent recovery	98.1	100.3

Thyroxine determination. In most instances 20 g. of iodinated casein was added to a hot solution containing 64 g. anhydrous $Ba(OH)_2$ dissolved in 128 ml. of distilled water. The hydrolysis was conducted in a 250-ml. boiling flask, attached to a reflux condenser by means of a ground glass joint. After the mixture was boiling smoothly, radioactive thyroxine stock solution was added, the condenser was connected, and the mixture was boiled gently for 18 to 20 hours.

The thyroxine was isolated and purified as described earlier *(12)*. It was then transferred to a tared, flat-bottomed Pyrex dish 22.0 mm. in diameter with the aid of distilled water, spread evenly over the bottom of the dish, dried to constant weight, and counted. Radioactivity counts were made with an end window G. M. tube connected to a laboratory scaler. Appropriate standards prepared from the same stock solution employed in the hydrolysis were placed on Pyrex dishes, dried, and counted with each series of determinations. The thyroxine content of the preparations was computed from the dilution of the radioactive thyroxine added during hydrolysis by nonradioactive thyroxine derived from the iodinated protein. The computation is that for simple isotope dilution as described by Kamen *(8)*.

Thyroxine content of thyroactive iodinated casein. The results of analyses of seven different samples of Protamone are given in Table 2. The data listed under Stage 1 are the values obtained after the isolated thyroxine had been purified to the point where microscopic examination revealed a homogeneous crop of characteristic "wheat bundle" crystals. After drying, weighing, and counting at this stage, the thyroxine was purified by further recrystallization (Stage 2). Preparations No. 1 through 5 were recrystallized once from Na_2CO_3 solution and once from boiling 70% alkaline alcohol. Preparations 6 and 7 were recrystallized first from Na_2CO_3 solution, and the crystals of the monosodium salt were washed four times with cold distilled water. After recrystallizing from hot alkaline alcohol they were washed again with three changes of cold distilled water and then crystallized alternately again from Na_2CO_3 and hot alkaline alcohol. Finally, the samples were dried to constant weight and recounted. The lack of any significant change in computed thyroxine content at the two stages indicates that little further purification could be attained by the methods employed. The

TABLE 2

Comparison of apparent and true thyroxine content of thyroactive iodinated casein as determined by n-butanol extraction and isotope dilution methods

Prep. No.	Thyroxine equiv. BuOH ext.	Isotope dilution analysis[b]							
		Labeled thyroxine added		Thyroxine recovered				Computed thyroxine content	
				Stage 1		Stage 2			
		Mg.	Sp. Act.[a]	Mg.	Sp. Act.	Mg.	Sp. Act.	1	2
	(%)							(%)	(%)
1	2.75	9.43	5.46	60.9	0.191	51.9	0.206	1.30	1.20
2	3.67	9.43	5.46	13.2	0.239	11.4	0.229	1.03	1.08
3	3.38	9.43	5.46	28.5	2.227	24.7	0.226	1.09	1.09
4	3.52	9.43	5.46	14.8	0.263	13.2	0.221	0.93	1.12
4	3.52	9.43	5.46	21.2	0.215	16.3	0.202	1.15	1.23
5	2.83	9.43	5.46	34.4	0.245	30.7	0.226	1.00	1.09
6	3.24	1.00	78.94	30.7	0.426	13.2	0.378	0.92	1.04
7	3.30	1.00	78.94	22.3	0.425	6.6	0.431	0.92	0.91
7	3.30	1.00	78.94	11.4	0.455	3.5	0.370	0.86	1.06
Mean	3.24							1.04	1.09
Std. error	0.429							0.049	0.033

[a] Counts per mg. per second corrected for background and physical decay.
[b] A 20-g. sample was analyzed in each case.

weighted mean true thyroxine content was 1.04 ± 0.049 at Stage 1 and 1.09 ± 0.033% at Stage 2. This compares to an apparent thyroxine content of 3.24 ± 0.429% by the butanol extraction method. It is of interest that the true thyroxine shows less variability between preparations than does the n-butanol-extractable fraction.

DISCUSSION

The results obtained indicate that only about one-third of the apparent thyroxine content of iodinated casein, as determined by the method of Reineke *et al. (13)* is true thyroxine. Three principal sources of error could conceivably affect the results obtained by the isotope dilution method described in this report. If exchange were to occur between I^{131} in the labeled thyroxine and iodide derived from the iodinated protein during the hydrolysis, very serious errors would result. The data presented in Table 1 indicate that no significant exchange occurs under the conditions employed. If either the labeled thyroxine or the thyroxine isolated from the hydrolyzate contained a significant amount of contaminants, errors would arise. In the first case the computed values would tend to be too low and in the second, too high. For this reason extreme care was taken in purifying the thyroxine at both of these stages. One-dimensional paper chromatograms run on some of the thyroxine isolated after hydrolysis indicated only one spot (Ninhydrin test), with no evidence of significant contaminants. It is probable that trace amounts of compounds similar to thyroxine in solubility were carried along in the purifications, but not in sufficient amounts to introduce a gross error.

The average thyroxine value of 1.04% obtained in the present study compares with a maximum yield from a similar preparation by direct isolation *(12)* of 0.424%. Even though isolation of thyroxine entails substantial losses during

purification, Pitt-Rivers *(9)* has reported that the yields obtained on products of varying potency agree closely with their biological activity. As pointed out in the review by Pitt-Rivers *(10)*, all of the analytical methods for thyroxine lack specificity when applied to iodinated proteins. The principal interfering substances for most of the methods appear to be diiodothyronine and triiodothyronine *(2, 3)*. These are eliminated by fractional crystallization in the isotope dilution technique.

Inasmuch as triiodothyronine is reported to exert 3.5 times the thyroidal action of L-thyroxine *(6)*, it does not seem valid to transpose the results of a chemical assay into expected biological potency at the present time. However, if the results of Frieden and Winzler *(5)* are re-evaluated on the basis that only one-third of the n-butanol-soluble fraction from iodinated casein hydrolyzates represents true thyroxine, the biological potencies of thyroid powder and iodinated casein, relative to their thyroxine content, are brought into much closer agreement.

SUMMARY

A procedure is described for the determination of thyroxine in thyroactive iodinated proteins by an isotope dilution technique employing radioactive thyroxine. It is shown that during hydrolysis in 40% barium hydroxide solution and subsequent isolation no significant exchange of iodine occurs between radioactive thyroxine and iodide. Analyses on seven different samples yielded an average true thyroxine value of 1.04 ± 0.049%. The average apparent thyroxine content (n-butanol soluble fraction) was 3.24 ± 0.429%. The "true" thyroxine thus comprised roughly one-third of the apparent thyroxine value.

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REFERENCES

(1) BLAXTER, K. L., REINEKE, E. P., CRAMPTON, E. W., AND PETERSEN, W. E. The Role of Thyroidal Materials and of Synthetic Goitrogens in Animal Production and an Appraisal of Their Practical Use. *J. Animal Sci.*, **8**: 307. 1949.

(2) BORROWS, E. T., CLAYTON, J. C., AND HEMS, B. A. The Synthesis of Thyroxine and Related Substances. Part III. The Synthesis of Thyroxine from 2,6-dinitrodiphenyl Ethers. *J. Chem. Soc.*, p. 199. 1949.

(3) FRIEDBERG, W., AND REINEKE, E. P. Thyroxine-like Compounds in Iodinated Casein and Thyroid Gland. *Federation Proc.*, **11**: 50. 1952.

(4) FRIEDEN, EARL, LIPSETT, M. B., AND WINZLER, R. J. Methods for Labelling Thyroxine with Radioactive Iodine. *Science*, **107**: 353. 1948.

(5) FRIEDEN, EARL, AND WINZLER, R. J. Comparative Parenteral Thyroxine-like Activity of Natural and Synthetic Thyroproteins Studied with the Goiter Prevention Method. *Endocrinology*, **43**: 40. 1948.

(6) HEMING, A. E., AND HOLTHAMP, D. E. Calorigenic and Antigoitrogenic Actions of L-triiodothyronine and L-thyroxine in Thyroidectomized and Intact Rats. *Proc. Soc. Exptl. Biol. Med.*, **83**: 875. 1953.

(7) HIRD, J. F. R., AND TRIKOJUS, V. M. Paper Partition Chromatography with Thyroxine and Analogues. *Australian J. Sci.*, 10: 185. 1948.

(8) KAMEN, MARTIN D. *Radioactive Tracers in Biology*, p. 117. Academic Press, Inc. New York. 1947.

(9) PITT-RIVERS, ROSALIND. The Chemical Assay of Biologically Active Iodinated Proteins: Isolation of Thyroxine. *Biochim. et Biophys. Acta*, 3: 675. 1949.

(10) PITT-RIVERS, ROSALIND. The Chemical Assay of Thyroxine and Other Substances with Thyroid Activity. In *Hormone Assay*, Edited by C. W. Emmons, Academic Press, Inc., New York City. 1950.

(11) REINEKE, E. P., AND TURNER, C. W. Formation in Vitro of Highly Active Thyroproteins, Their Biologic Assay, and Practical Use. Mo. Agr. Expt. Sta., *Research Bull.* 355. 1942.

(12) REINEKE, E. P., AND TURNER, C. W. The Recovery of Crystalline Thyroxine from Iodinated Casein. *J. Biol. Chem.*, 149: 555. 1943.

(13) REINEKE, E. P., TURNER, C. W., KOHLER, G. O., HOOVER, R. D., AND BEEZLEY, M. B. The Quantitative Determination of Thyroxine in Iodinated Casein Having Thyroidal Activity. *J. Biol. Chem.*, 161: 599. 1945.

(14) SYKES, J. F., ANDREWS, F. N., HILL, F. W., LORENZ, F. W., THOMAS, J. W., AND WINCHESTER, C. F. Hormonal Relationships and Applications in the Production of Meats, Milk and Eggs. *National Research Council Publication*, 266. 1953.

THE EFFECTS OF VARIED RATES OF HAY FEEDING ON BODY WEIGHT AND PRODUCTION OF LACTATING DAIRY COWS[1]

T. G. MARTIN, G. E. STODDARD,[2] AND R. S. ALLEN
Iowa Agricultural Experiment Station, Ames

Many levels of roughage feeding have been reported. Mead and Goss *(10)* reported that cows fed concentrates alone were more subject to bloat than animals fed hay plus concentrates, but otherwise the well-being of the animals was not affected. At the other extreme, Sherwood and Dean *(15)* observed that animals fed hay alone did not suffer harmful effects but produced less milk than cows given a supplement of grain. Smith *et al. (16)* observed that cows fed hay alone did not produce as much milk as would be expected from the TDN (total digestible nutrient) evaluation of the ration. In a review, Huffman *(4)* stated that the quality and productive energy content of hays are highly variable and that hay does not always yield production results comparable to TDN content. Huffman and Duncan *(5, 6, 7, 8)* and Huffman *et al. (9)* presented data indicating that various concentrate feeds, when substituted on an equal TDN basis for part of the hay in the ration of a lactating cow previously fed alfalfa hay alone, caused increased milk production. As a result of these findings, it was postulated that unknown milk production factors which caused increased production were present in the concentrate material.

Moore *et al. (11)* and Saarinen *et al. (11)* have suggested that the increased production attributable to substitution of grain for hay on an equal TDN basis can be accounted for by an increased productive energy or net energy content of the ration. Davis *et al. (2)* attributed the observed increased production to increased productive energy content of the ration rather than to "unidentified factors."

The reports cited above have, for the most part, centered around the study of hay-grain replacement at high and low levels of hay feeding. The experiment reported here was designed to determine whether or not varying the rate of hay feeding within normal limits while maintaining a constant TDN intake has an effect on the level of production or the well-being of the animals.

EXPERIMENTAL PROCEDURE

Twenty Holstein cows were used in two trials to evaluate four levels of alfalfa hay feeding. Rates of hay feeding were established at 0.50, 1.17, 1.83, and 2.50 lb. of hay daily per 100 lb. body weight and were designated rations A, B, C, and D, respectively. During a 2-week preliminary period, all cows were

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[2] Present address: Dairy Industry Department, Utah State Agricultural College, Logan.

fed hay at the daily rate of 2 lb. per 100 lb. body weight. Rate of concentrate feeding during the preliminary period was established at a level sufficient to supply 100% of Morrison's *(12)* recommended levels for average production and maintenance of each cow. In order to have most of the cows at the peak or in the declining phase of lactation, no cow was placed on the preliminary period of the experiment less than 30 days after freshening. The range of days after freshening was from 30 to 139.

Initial ration calculation for the experimental period was based on the level of production and average weight during the preliminary period. Thereafter, concentrate allowance was reduced at the monthly rate of 6% of total TDN allowance for milk production. The reduction was calculated at the end of each 4-week period in the continuous trial and at the end of each 3-week period on the crossover trial. The above rate of reduction was in accordance with the findings of Gaines *(3)*. Hay was fed at the assigned level and was supplemented by sufficient concentrate mixture to supply total TDN (from hay and concentrates) at 100% of Morrison's recommended levels. Composition of the concentrate mixture was: ground corn, 50 parts; ground oats, 35 parts; soybean oil meal, 15 parts; steamed bone meal, 2 parts; and salt, 1 part. The hay had an average proximate analysis of: fiber, 30.3%; protein, 11.7%; ether extract, 1.5%; ash, 6.0%; nitrogen-free extract, 35.3%; and moisture, 15.2%.

TDN contents of the concentrate mixture and of the hay were calculated from proximate feed analyses made during the course of this experiment and digestibility coefficients taken from Morrison. Values calculated in the above manner varied considerably from average values presented by Morrison. However, adjustment of the calculated values to the moisture content of the comparable feeds listed by Morrison brought the estimates and Morrison's values into very close agreement. ENE (estimated net energy) value of the concentrate mixture and hay was computed by adjusting Morrison's ENE values to the moisture content of the feeds used in this study.

The 20 cows used in these trials were divided into five groups according to freshening date so that the stage of lactation of the four cows in each group was as uniform as possible. One group of four cows was assigned to a 4×4 Latin-square design trial wherein each cow received each ration during one of the four 3-week periods and no one ration appeared more than once during any given period. This trial was designated the crossover trial. One cow from each of the remaining four groups was assigned to each of the four rations and all cows were maintained on the assigned ration for a 16-week period. This trial was designated as the continuous feeding trial.

Cows were weighed once weekly and also on three consecutive days when an animal was changed from one ration to another and when placed on or taken off experiment. Daily milk weights were recorded for all cows and milk-fat analyses were obtained weekly and bi-weekly for cows assigned to the crossover and continuous trials, respectively. Milk production data were converted to FCM (4% fat-corrected values).

Protein and dry matter digestibilities were determined by the chromogen

method of Reid *et al. (13)*. Grab samples of feces were collected daily for periods of at least 2 days. Computation of approximate digestibility values was based on average analytical data for samples collected in each period. Samples were collected during each period from each cow on the crossover trial and in the fourth month of the experimental period from each cow on the continuous trial. Dry matter and protein analyses were determined by A.O.A.C. methods *(1)*. Reliability of the digestibility data is somewhat limited by the fact that the chromogen content of the alfalfa hay was based on a limited number of analyses.

Statistical treatment of the data was accomplished by methods described by Snedecor *(17)*.

RESULTS AND DISCUSSION

Body weights of cows on the crossover trial were variable and followed no set pattern. An analysis of variance of these data revealed no significant effect of ration on body weight. In the same trial, milk production (FCM) tended to decrease as rate of hay feeding increased. The actual data are presented in Table 1 and the analysis of variance in Table 2. The significant effect of rations on

TABLE 1

Average daily FCM produced by cows on the crossover trial

Period	Cow No. 3142	2956	3129	2963	Period Av.
Preliminary	38.9	40.7	49.0	48.1	44.2
I	A[a] 37.5	D 32.6	C 42.1	B 42.7	38.7
II	B 33.4	A 32.8	D 36.5	C 40.2	35.7
III	C 27.8	B 29.2	A 39.7	D 32.0	32.2
IV	D 26.9	C 27.6	B 36.6	A 37.1	32.1
Cow av. (expt. per. only)	31.4	30.6	38.7	38.0	
Ration average	A 36.8	B 35.5	C 34.4	D 32.0	

[a] Rates of hay feeding on the various rations were: A, 0.50; B, 1.17; C, 1.83; and D, 2.50 lb. hay per cwt. body weight daily.

TABLE 2

Analysis of variance of milk production data in crossover trial

Source of variance	Degrees of freedom	Mean squares	F
Cows	3	73.67	
Periods	3	40.67	
Rations	3	16.33	13.06**
Error	6	1.25	

** $P \leq 0.01$

milk production is subject to some question, inasmuch as ration calculation was based on a TDN evaluation of hay which was analyzed at the beginning of the experiment. It was found, however, that hay samples collected during the trial had lower TDN values. Thus, the analyses of all hay samples were averaged and the mean TDN value was used in computing TDN available for milk production by the following formula:

Available TDN = total TDN − (maintenance TDN + feed refusal TDN). ENE available for production was computed in a like manner. Interpretations presented in this report were based on the available TDN and ENE values.

It seemed reasonable that the decline of milk production as hay was increased should be correlated with the decline of TDN intake (crossover trial section of Table 3). An analysis of covariance was computed wherein the milk production values were adjusted to a common level of TDN. Adjusted ration averages

TABLE 3
Average TDN and ENE (available for production) values

Trial	Energy measure	Ration[a]			
		A	B	C	D
Crossover	TDN[b]	13.34	13.05	12.84	12.45
	ENE[c]	14.58	13.74	12.99	12.04
Continuous	TDN	12.54	11.67	11.51	12.79
	ENE	13.72	12.30	11.62	12.44

[a] Rates of hay feeding on the various rations were: A, 0.50; B, 1.17; C, 1.83; and D, 2.50 lb. hay per cwt. body weight, daily.
[b] Average lb. per day per cow.
[c] Average therms per day per cow.

ranged from 34.5 to 34.8 lb. FCM per day, and there was no significant effect of ration on milk production. The result of this test of significance would infer that, had the rations actually been calculated on a constant TDN basis, there would have been no significant ration effects on milk production. A similar adjustment to a common ENE basis also removed the significant effect of rations on milk production.

The continuous trial was divided into four periods of 4 weeks each, and the weight change data were analyzed in each period separately as well as over the entire 16-week period. No significant differences among rations were found.

Since cows were assigned to groups according to stage of lactation, the range of producing ability was large both in group and in ration classifications. As a result, the analysis of variance of milk production data, which are summarized in top section of Table 4, did not yield any significant effects of ration on milk production. Because of the fact that production in the preliminary period was highly correlated with production during the experimental period (see Table 5), the removal of cow differences by adjusting the experimental period data to a common preliminary period level of production seemed justified. The adjusted ration averages are presented in the center section of Table 4. Tests of significance were made by analysis of covariance and a significant ($P \leq 0.05$) effect of ration on milk production was found in the first 4-week period and in the entire

TABLE 4

Average daily FCM produced by cows on the continuous trial

Type observation	Ration[a]	Period I	Period II	Period III	Period IV	Ration Av.
Actual production FCM	A	38.2	36.8	31.0	27.9[b]	33.5
	B	36.1	33.0	31.3	29.2	32.5
	C	34.8	29.5	27.4	25.3	29.2
	D	34.4	31.1	29.7	26.6	30.4
	Period Av.	35.9	32.6	29.8	27.2	
FCM adjusted[c] for variation in preliminary period prod.[d]	A	29.1	37.7	31.9	28.5	34.4
	B	37.9	34.9	33.1	30.6	34.2
	C	35.0	29.7	27.6	25.4	29.4
	D	31.4	28.0	26.6	24.2	27.4
	Period Av.	35.8	32.6	29.8	27.9	
FCM adjusted[c] for variation of TDN available for prod.	A	37.2	36.0	29.6	26.5	32.5
	B	37.1	34.3	32.8	30.2	33.6
	C	36.2	31.2	28.6	25.5	30.8
	D	33.0	27.1	27.6	26.1	28.8
	Period Av.	35.9	32.2	29.7	27.1	

[a] Rates of hay feeding on the various rations were: A, 0.50; B, 1.17; C, 1.83; and D, 2.50 lb. hay per cwt. body weight daily.

[b] Average of only 3 observations.

[c] Adjustments based on regressions of FCM on preliminary period production and TDN available for production, respectively. Ration averages and period averages adjusted independently.

[d] Average daily preliminary period production (lb. FCM) of cows placed on various rations was: A, 39.5; B, 38.4; C, 40.4; D, 44.3.

TABLE 5

Correlations[a] of average daily FCM production, preliminary period production, and TDN available for milk production during experimental periods

Correlation	Period I	Period II	Period III	Period IV	Entire expt. period
FCM and prelim. prod.	0.958	0.874	0.895	0.930	0.930
FCM and TDN	0.920	0.874	0.896	0.893	0.908
TDN and prelim. prod.	0.963	0.959	0.956	0.963	0.964

[a] Correlations computed from error sums of squares and crossproducts.

experimental period. There was no significant effect of ration on production during the second, third and fourth 4-week periods.

Adjustment for preliminary period production did not correct the effect of declining TDN as hay content of the ration increased. Since ration calculation was based on preliminary period production, TDN available for production was highly correlated (see Table 5) with preliminary period production. Therefore, adjustment of milk production data to a common level of TDN available for milk production (continuous trial ration averages in Table 3) largely corrected for variability of producing ability of cows as well as variability of TDN available for milk production. Ration averages adjusted in this manner are shown in the bottom section of Table 4. There were no significant effects of ration on milk production when all rations were on a common TDN basis. Adjustment to a common level of ENE available for milk production yielded similar results.

Since the reliability of the digestibility data was subject to some question, no

attempt was made to correlate digestion of nutrients with level of production or weight gain or loss. The relationship of digestibility to per cent hay in the ration, though not surprising, seemed interesting. Regression equations of the lines

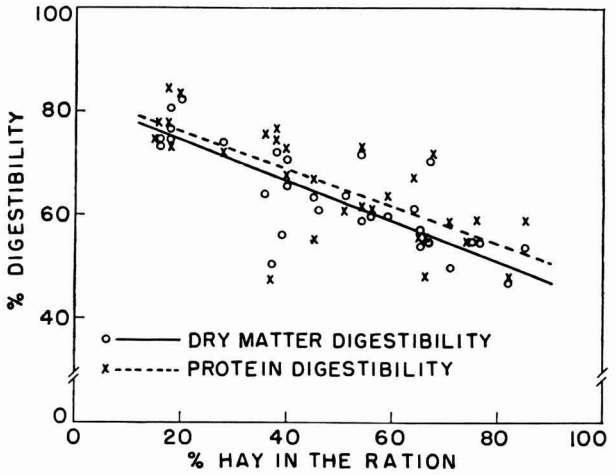

FIG. 1. Distribution and regressions of dry-matter and protein digestibility data relative to per cent hay in the ration.

shown in Figure 1 are: $\hat{Y} = 82.9 - 0.35\ X$ and $\hat{Y} = 82.2 - 0.38\ X$ for protein and dry matter digestibilities, respectively, where Y = per cent digestibility and X = per cent hay in the ration.

The coefficient of correlation of dry matter digestibility with per cent hay in the ration was 0.82 in both trials and in the pooled data. The coefficients of correlation of protein digestibility with per cent hay in the ration were 0.82 and 0.71 in the crossover and continuous trials, respectively, and 0.71 when both trials were pooled.

Inasmuch as both TDN and ENE (available for milk production) values were computed in the course of analyzing these data, it seemed of interest to know *(a)* to what degree they were correlated with milk production, *(b)* whether or not one was a more consistent estimator of the value of a ration over a wide range of hay: concentrate ratios, and *(c)* whether or not weight change was correlated with either milk production or efficiency of production.

In order to get as many observations as possible to study the above questions, all data from both trials and the preliminary period were considered. TDN and ENE (available for milk production) were highly correlated with milk production, the correlation coefficients being 0.941 and 0.942, respectively.

An index to efficiency of a ration was considered to be lb. milk per lb. TDN or lb. milk per therm ENE. If either TDN or ENE could be considered a consistent estimator of the value of a ration, the regression of the efficiency index of that estimator on the per cent hay in the ration should be nonsignificant.

Computation of these regressions on the total data and on each trial separately yielded no consistent pattern. It appeared, however, that ENE was somewhat more consistent as an estimator of the value of a ration over a wide range of hay:concentrate ratios.

Correlation of weight change and milk production was nonsignificant and near zero. Correlation of weight change and efficiency of milk production (based on either index) was also nonsignificant and near zero.

SUMMARY

Twenty lactating Holstein cows were used in two trials to test the effects of feeding hay at levels of 0.50, 1.17, 1.83, and 2.50 lb. per 100 lb. body weight.

There were no significant effects of level of hay feeding on body weight change. If TDN or ENE was held constant, there were no significant effects of level of hay feeding on milk production.

Body weight change was not correlated with either milk production or efficiency of production. TDN available for milk production was highly correlated with milk production (coefficient of correlation 0.941). ENE available for milk production had a coefficient of correlation of 0.942 with milk production.

Based on this experiment, neither TDN nor ENE could definitely be said to be superior to the other as an estimator of the worth of a ration, though ENE did seem to be somewhat more consistent over a wide range of hay:concentrate ratios.

Both protein and dry matter digestibility values declined as hay content of the ration increased.

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REFERENCES

(1) Association of Official Agricultural Chemists. *Official Methods of Analysis*, 7th ed. The Association, Washington, D. C. 1950.

(2) Davis, R. F., Loosli, J. K., and Warner, R. G. Are There "Unidentified Lactation Factors" for Cows? *J. Dairy Sci.*, 36: 581. 1953.

(3) Gaines, W. L. Persistency of Lactation in Dairy Cows, a Preliminary Study of Certain Guernsey and Holstein Records. Ill. Agr. Expt. Sta. *Bull.* 288. 1927.

(4) Huffman, C. F. Roughage Quality and Quantity in the Dairy Ration, a Review. *J. Dairy Sci.*, 22: 889. 1939.

(5) Huffman, C. F., and Duncan, C. W. The Nutritive Value of Alfalfa Hay. II. Starch and Glucose as Supplements to an All-Alfalfa Hay Ration. *J. Dairy Sci.*, 27: 821. 1944.

(6) Huffman, C. F., and Duncan, C. W. The Nutritive Value of Alfalfa Hay. III. Corn as a Supplement to an All-Alfalfa Hay Ration for Milk Production. *J. Dairy Sci.*, 32: 465. 1949.

(7) Huffman, C. F., and Duncan, C. W. The Nutritive Value of Alfalfa Hay. IV. Beet Pulp, Corn Gluten Meal and Soybean Oil Meal as Supplements to an All-Alfalfa Hay Ration for Milk Production. *J. Dairy Sci.*, 33: 710. 1950.

(8) Huffman, C. F., and Duncan, C. W. Unidentified Dietary Factors in Dairy Cattle Nutrition. I. Digestibility of Peanut Hulls and Their Use in "Ballast" Studies with Milking Cows Depleted on Hay Alone. *J. Dairy Sci.*, **35**: 30. 1952.

(9) Huffman, C. F., Duncan, C. W., and Chance, C. M. Unidentified Dietary Factors in Dairy Cattle Nutrition. II. Further Evidence of an Unidentified Factor(s) in Grain Needed to Balance Roughage for Milk Production. *J. Dairy Sci.*, **35**: 41. 1952.

(10) Mead, S. W., and Goss, H. Ruminant Digestion Without Roughage. *J. Dairy Sci.*, **18**: 163. 1935.

(11) Moore, L. A., Irvin, H. M., and Shaw, J. C. Relationship between TDN and Energy Value of Feeds. *J. Dairy Sci.*, **36**: 93. 1953.

(12) Morrison, F. B. *Feeds and Feeding*, 21st ed. Morrison Publishing Co., Ithaca, N. Y. 1951.

(13) Reid, J. T., Woolfolk, P. G., Richards, C. R., Kaufmann, R. W., Loosli, J. K., Turk, K. L., Miller, J. I., and Blaser, R. E. A New Indicator Method for the Determination of Digestibility and Consumption of Forage by Ruminants. *J. Dairy Sci.*, **33**: 60. 1950.

(14) Saarinen, P., Sami, M. A., Shaw, J. C., and Moore, L. A. The Adequacy of an All-Alfalfa Hay Ration for Milk Secretion. *J. Dairy Sci.*, **34**: 287. 1951.

(15) Sherwood, D. H., and Dean, H. K. Feeding Alfalfa Hay Alone and with Concentrates to Dairy Cows. Ore. Agr. Expt. Sta. *Bull.* 380. 1940.

(16) Smith, V. R., Jones, I. R., and Haag, J. R. Alfalfa with and without Concentrates for Milk Production. *J. Dairy Sci.*, **28**: 343. 1945.

(17) Snedecor, G. W. *Statistical Methods*. Iowa State College Press, Ames, Iowa. 1946.

SOME FACTORS AFFECTING THE ACTION OF BENZOYL PEROXIDE IN THE BLEACHING OF MILK AND CREAM FOR BLUE CHEESE MANUFACTURE[1, 2]

S. KURAMOTO AND J. J. JEZESKI

Department of Dairy Husbandry, University of Minnesota, St. Paul

The color of the curd of blue-veined cheese will vary depending on the season of manufacture. Cheese made during the winter months will have a light background color similar to Roquefort, whereas cheese manufactured during summer months will exhibit a yellowish color because of the higher level of carotene in the fat. Federal food and drug regulations *(2)* permit the use of benzoyl peroxide for the purpose of bleaching milk for the manufacture of certain types of cheese; and since it is desirable to maintain the light color similar to Roquefort cheese, bleaching with benzoyl peroxide affords a possible means of effectively destroying the carotenoid pigments of milk and controlling the color of the cheese.

Very little information is available in the literature either as to the factors affecting the bleaching of the carotenoid pigments of milk or possible defects that may arise through the use of benzoyl peroxide. Hunziker *(3)* has cited an incident where benzoyl peroxide was used in bleaching cream for butter making. Although no references were presented, he claimed that the destructive effect of this peroxide on the glycerides and vitamin A prevented its widespread use in the manufacture of a light-colored butter. Other information available deals with the use of benzoyl peroxide in the bleaching of flour and also in the chemical decolorization of inedible fats and tallows.

This study was undertaken to investigate some of the factors involved in the bleaching of the carotenoid pigments of milk by the use of benzoyl peroxide. The effect of bleaching time, temperature, and concentration of bleaching agent were studied to establish some conditions under which milk may be processed for Blue cheese manufacture.

METHODS

The benzoyl peroxide used is marketed under the trade name of "Novadelox," a product more commonly used in the bleaching of flour. Because of the explosiveness of the reagent, it is compounded with $CaSO_4$ and $MgCO_3$, which make it a relatively safe compound of low heat sensitivity and one that will not cake when stored. The active peroxide in this product comprises 16% of the total weight of powder.

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[1] Supported in part by the Felix and Dorothy Frederiksen Cheese Research Fund.

[2] The data in this paper are from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Master of Science, University of Minnesota. Scientific Journal Series Paper No. 3155, Minnesota Agricultural Experiment Station.

In the bleaching process, the milk was treated as follows:

1. The milk was first separated to produce cream containing 30% fat, unless otherwise stipulated.

2. After preheating the milk or cream to the desired temperature, an aqueous 10% suspension of Novadelox was added, the amount being calculated on a weight basis of the concentration of active benzoyl peroxide desired in 4% milk.

3. The cream was bleached at temperatures ranging from 125 to 185° F. for periods up to 4 hours with concentrations of 0.00045, 0.0009, and 0.0018% benzoyl peroxide.

Carotenoid and vitamin A determinations were made by the spectrophotometric method of Boyer *et al. (1)*. Carotene was determined first from a petroleum ether extraction of the unsaponifiable fraction of milk, and vitamin A by the Carr-Price reaction with $SbCl_3$. A differential carotenoid separation was not performed; consequently total carotenoid values are reported. Carotenoid values were obtained from a standard curve obtained with crystalline α, β, carotene. Vitamin A values were obtained similarly with crystalline vitamin A alcohol as a reference. Vitamin A and carotenoid values are expressed in terms of International Units (I.U.) per pound of fat. One I.U. was taken as being equivalent to 0.3 and 0.6γ of vitamin A and carotene, respectively. Flavor scores were determined organoleptically by three experienced judges, and particular attention was directed toward oxidized and tallowy flavors resulting from the bleaching process.

RESULTS

Bleaching cream with benzoyl peroxide did not appear to have an appreciable effect on the vitamin A content of butterfat even though bleaching of the carotenoid pigments was observed. Figure 1 presents the carotenoid and vitamin A values obtained when cream was bleached at temperatures of 125° and 145° F. for periods up to 4 hours and at a benzoyl peroxide concentration of 0.0009%.

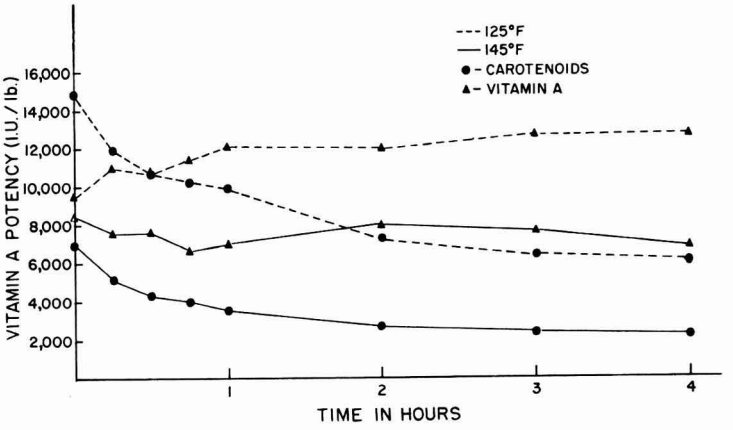

FIG. 1. Vitamin A and carotenoid values of 30% cream bleached over a period of 4 hours at 125° and 145° F. with 0.0009% benzoyl peroxide.

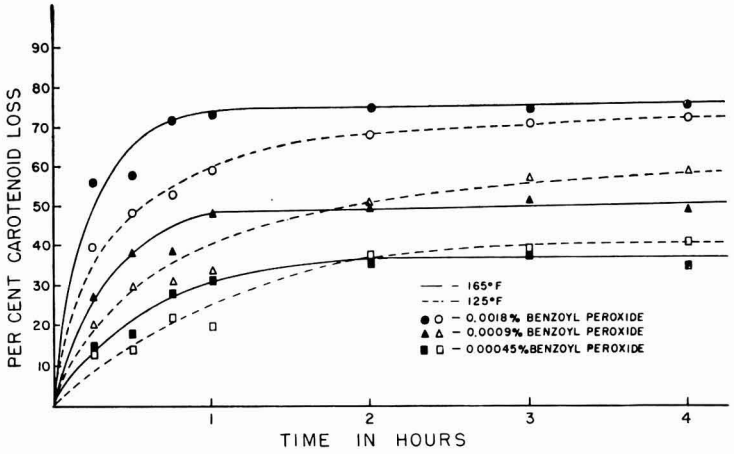

FIG. 2. The influence of concentration of bleaching agent and bleaching time on the percentage loss of carotenoids in cream bleached at 125° and 165° F.

Figure 2 shows the percentage loss of carotenoids as a function of time. It is apparent that temperature affects the rate of carotenoid loss, since at 125° F. carotenoid losses did not reach maximum values until after 2 to 3 hours, whereas at 165° F. bleaching was much faster and maximum loss was reached at the end of 1 hour. An increase in temperature did not markedly affect the total percentage loss of carotenoids. In general, at the two temperatures used, maximum carotenoid destruction of approximately 35, 50, and 75% resulted when 0.00045, 0.0009, and 0.0018% benzoyl peroxide concentrations were used, respectively. It was significant that irrespective of the initial carotenoid content of cream prior to bleaching, the percentage loss of carotenoids at a given concentration of benzoyl peroxide was constant. Whereas increases in temperature hastened the relative rate of carotenoid destruction, total percentage loss of milk carotenoids appeared to be controlled by the concentration of benzoyl peroxide used.

In all cases where 0.0018% benzoyl peroxide was used, oxidized and tallowy flavors resulted. Despite the excellent carotenoid destruction observed with 0.0018% of the reagent, the development of the tallowy flavors indicated that this concentration was excessive at all temperatures studied. Bleaching temperatures of 165° and 185° F. were investigated, but the development of intense cooked and scorched flavors with all concentrations of benzoyl peroxide tested made those temperatures undesirable.

Bleaching cream at temperatures of 125° and 145° F. resulted in the production of least off-flavors. However, the use of 0.00045% benzoyl peroxide at these latter temperatures produced somewhat ineffective bleaching, as only about 30% destruction of carotenoids was observed. The use of 0.0009% peroxide at temperatures of 125° and 145° F. for periods of up to 2 hours gave the most efficient bleaching from the over-all standpoint of carotenoid destruction and minimum development of undesirable flavors. Table 1 presents the oxidized flavor intensi-

TABLE 1

Oxidized flavor intensity in cream bleached at 125° and 145° F. with 0.0009 and 0.0018% benzoyl peroxide

Time	125° F.		145° F.	
	0.0009%	0.0018%	0.0009%	0.0018%
(hr.)				
0	−	−	−	−
¼	+	++	++	++
½	++	++	++	++
¾	+	++	++	+
1	−	++	++	++
2	−	++	++	++++
3	−	+++	+	++++
4	−	++++	+	++++

Key: + slight oxidized; ++ oxidized; +++ strong oxidized; ++++ tallowy.

ties of cream bleached at 125° and 145° F. when 0.0009% and 0.0018% benzoyl peroxide were used. At 125° F. and with 0.0009% reagent, off-flavors of mild intensity were observed early in the bleaching period but disappeared on continued heat exposure. These were rather atypical flavors possessing a "nutty" characteristic which was thought to be due to the unexpended peroxide.

A greater percentage of carotenoid loss was observed when the fat percentage of samples was increased (Figure 3). Maximum values of about 78, 50, 38, and 15% carotenoid destruction occurred when samples containing 50, 30, 10, and 4.5% butterfat, respectively, were bleached. One might expect no change in bleaching action between high and low fat samples since the ratio of fat to benzoyl peroxide was the same in all cases. If one considers that the reagent becomes entirely dissolved in the fat, then the fat percentage of the cream should not affect the extent of bleaching. However, if the reagent remains suspended in

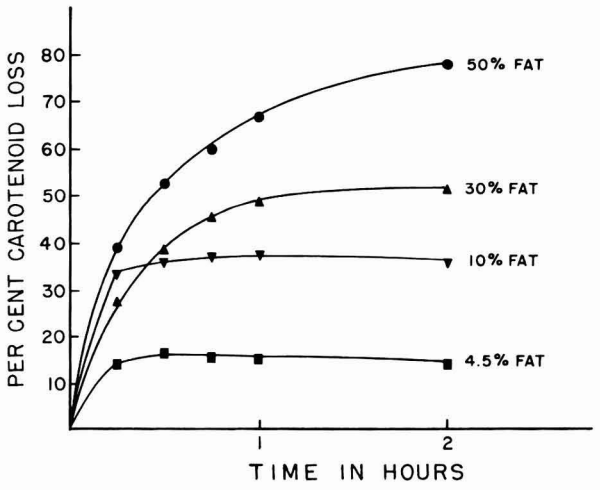

FIG. 3. The relationship between the percentage of carotenoid loss, time, and the fat content of the sample being bleached at 165° F. with 0.0009% benzoyl peroxide.

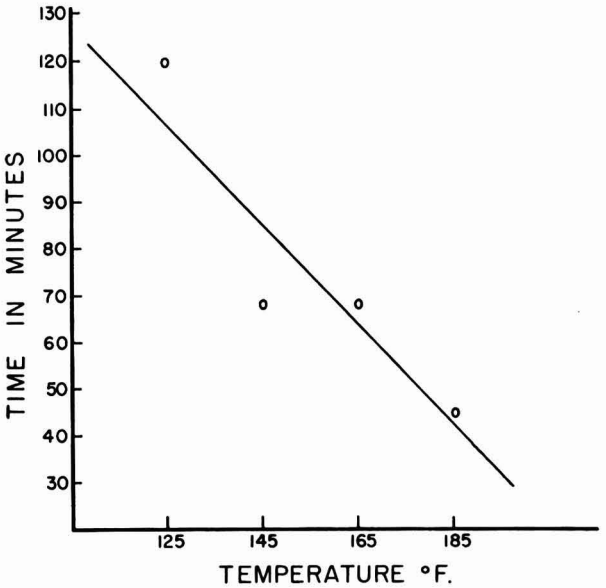

FIG. 4. The time-temperature relationship to produce 50% carotenoid loss in 30% cream with 0.0009% benzoyl peroxide.

the skimmilk, the relative concentration of bleaching agent increases as the fat percentage rises. It was apparent that the cream with a higher fat test was bleached to a greater extent. This might be taken as evidence that the reagent may not have been dissolved completely in the fat.

Fifty per cent carotenoid loss in summer milk was assumed to result in a level of carotene comparable to winter milk *(4, 5)*. Figure 4 shows the relationship between time and temperature required to attain a 50% carotenoid loss when 0.0009% benzoyl peroxide was used. The regression line representing the various time and temperature requirements for 50% carotenoid loss was the result of a statistical analysis of the data. This line indicates that, when using 0.0009% benzoyl peroxide, times from 45 minutes at 185° F. to 120 minutes at 125° F. were adequate in attaining 50% carotenoid loss.

DISCUSSION

Federal regulations permitting the use of benzoyl peroxide stipulate that the weight of the benzoyl peroxide used should not be more than 0.002% by weight of the milk being bleached. Furthermore, if milk is bleached in this manner, vitamin A must be added to the milk to compensate for the vitamin A or its precursor that may be destroyed during the bleaching process.

Apparently benzoyl peroxide was unable to destroy the vitamin A, since even under relatively vigorous conditions, no significant loss in vitamin A was observed. One would expect significant oxidation of the unsaturated vitamin A to

result from the action of an oxidizing agent such as benzoyl peroxide. In this respect, the differential destruction of assayable carotenoids and the relative ineffectiveness of the reagent toward the vitamin A was a curious phenomenon. Under certain conditions, there was some indication of a slight, but unexplainable, increase in vitamin A as the carotenoids disappeared.

It has been shown that bleaching can be performed efficiently under conditions that will prevent development of highly undesirable flavors in cream. Under certain times and temperatures of processing and keeping within the concentration limits prescribed by federal regulations, milk of acceptable quality for cheese making was found to result.

In order to attain a color of milk comparable to winter conditions, 50% carotenoid destruction was taken as an arbitrary end point to which bleaching should be carried. Time, temperature, and concentration interrelationships were found to exist. Temperatures from 125° to 145° F., benzoyl peroxide concentrations of 0.0009%, and bleaching periods from 1 to 2 hours were found to be adequate in obtaining the desired carotenoid destruction. Under these conditions, prolonged bleaching up to 2 hours appeared to improve the flavor without the concurrent development of undesirable oxidized and tallowy flavors. However, the development of oxidized flavors of mild intensity may not necessarily be detrimental, since these flavors apparently do not carry over into Blue cheese or else are masked by the more pungent flavors of the saturated short chain fatty acid compounds produced during the ripening process.

SUMMARY

Milk for Blue cheese manufacture may be bleached with benzoyl peroxide under conditions that will prevent the development of highly undesirable flavors. Using 50% carotenoid loss as an end point, cream treated at 125° and 145° F. with 0.0009% benzoyl peroxide for 90 to 120 minutes was sufficiently bleached, without the formation of objectionable oxidized and tallowy flavors. More efficient carotenoid decolorization could be effected by using cream with a higher fat content. Regardless of the original carotenoid level of raw cream, similar proportions of carotenoids were destroyed when a given concentration of benzoyl peroxide was used.

ACKNOWLEDGMENT

We wish to thank E. L. Thomas, H. A. Harland, and H. A. Morris for their assistance in grading cream samples.

REFERENCES

(1) Boyer, P. D., Spitzer, R., Jensen, C., and Phillips, P. H. Determination of Vitamin A and Carotene in Milk. *Ind. Eng. Chem., Anal. ed.*, **15**: 101. 1944.

(2) *Federal Register*. **15**: 5663. 1950.

(3) Hunziker, O. R. *The Butter Industry*, 3rd ed. Published by the author, LaGrange, Ill. 1940.

(4) Jenness, R., and Palmer, L. S. Vitamin A Potency of Creamery Butter Produced in Minnesota. *J. Dairy Sci.*, **28**: 473. 1945.

(5) Weswig, P. H., Haag, J. R., and Pierce, R. T. Seasonal Variation in the Vitamin A Potency of Oregon-Produced Blue Vein Cheese. *J. Dairy Sci.*, **34**: 649. 1951.

PEOPLE and EVENTS
in the Dairy Science World

Warnick Addresses Illinois Farmers

M. M. WARNICK, president of A.D.A., was the main speaker at the annual University of Illinois Dairy Day held on the Urbana campus Sept. 9. Mr. Warnick explained how A.D.A. is spending the money being raised by the dairy farmers in 43 states for advertising, merchandising, public relations, and research, all pointed in the direction of increased sales of milk and its products on a more profitable basis for the farmer. He emphasized the fact that A.D.A. does not operate in the field of legislation.

By this fall, A.D.A. will be advertising the dairy industry before an audience of 50 million people every week. Backing this up will be in-store merchandising, research on new products and on the condition of the dairy foods market, and public relations through magazines, newspapers, radio, and television. The whole advertising program is geared to the central idea, "you never out-grow your need for milk" and "drink three glasses daily."

Mr. Warnick emphasized the fact that dairy farming today is big business and isn't merely eking out an existence by milking a few cows as in past years.

Grassland Farming

A program on Grassland Farming sponsored by the Joint Committee on Grassland Farming and the Soil Conservation Society of America will be held at Jacksonville, Fla., Nov. 13-14, 1954. On Nov. 13, tours will be made to farms near Jacksonville, where different systems of grassland farming will be studied. The following program has been planned for Nov. 14.

Day Length and Crop Production—H. A. BORTHWICK, USDA, Beltsville, Md.

Potentialities of Coastal Plain Grasslands with Heavy Fertilization—G. W. BURTON, Georgia Coastal Plain Experiment Station and Univ. of Georgia, Tifton.

Lessons from Pasture Studies in Florida, Iowa, and Kansas (Tentative title)—G. B. KILLINGER, Univ. of Florida, Gainesville.

Trace Elements in Animal Nutrition in Florida—G. K. DAVIS, Univ. of Florida, Gainesville.

Year-Round Forage Program for Georgia—B. H. HENDRICKSON, Soil Conservation Service, Watkinsville, Ga.

The Range Cattle Industry in Florida—W. G. KIRK, Univ. of Florida Experiment Station, Ona.

The Dairy Industry of the South—HERMAN BOYD, president, Florida Dairy Association, Holl and Boyd Dairy Farms, Inc., Miami.

The Future of Beef Cattle Industry in the South (Tentative title)—IRLO BRONSON, Kissimmee, Fla., formerly president, Florida Cattlemen's Assoc., state senator, and cattle rancher.

Kentucky Events

The annual Univ. of Kentucky Animal Nutrition Conference is scheduled for Sept. 30 and Oct. 1, 1954. This conference is presented each year by the Departments of Animal Husbandry, Poultry Husbandry, Dairy Husbandry, and Feeds and Fertilizer. The meeting is especially for those interested in the manufacture, mixing, sale, and use of commercial feeds. An outstanding panel of off-campus speakers will assist the Univ. of Kentucky staff members in presenting new information regarding livestock feeds and feeding practices.

Kentucky dairymen in a referendum on August 21 rejected a program for a compulsory 12-month set-aside to promote the sale of dairy products. The program was to be administrated under a Dairy Commission consisting of six members vested by law with the authority to assess not to exceed 2 cents per hundred pounds of milk or ½ cent per pound of butterfat. Funds thus raised were to be used to stimulate research, advertising and merchandising of dairy products. The vote was close, with those opposed holding an advantage of about 10% in the ballots cast. As a result of the vote it is probable that Kentucky will continue on a voluntary 60-day set-aside for the A.D.A. program.

L. A. RICHARDSON, who received a Master's degree from the Univ. of Kentucky in August, has been appointed to fill a newly created position in the Agricultural Experiment Station. In his position as assistant in dairy technology he will devote his time principally to research in the field of cream quality improvement.

Preliminary plans for the 2nd Annual Dairy Manufacturing Short Course at the Univ. of Kentucky are taking form. The Dairy Products Association of Kentucky again will cosponsor this educational project. Dates selected are Nov. 9 and 10.

J. A. TAYLOR, who was granted a Master's degree at the Univ. of Kentucky in June, 1954, is now teaching dairying and managing the college dairy farm at Eastern State College, Richmond, Ky.

T. W. DENTON, who also received a Master's degree at the Univ. of Kentucky in June, 1954, has been granted a research assistantship at North Carolina State College.

C. L. DAVIS is now filling a staff appointment at U. K. as Assistant in Dairying. The appointment was effective June 1, 1954.

The following men are graduate students in dairy production at the University of Kentucky:

L. D. BROWN and MAURICE COLE, graduates of Western State College, Bowling Green, Ky.

G. F. FRIES, B.S., Washington State College, Pullman.

J. W. RUST, B.S., Univ. of Kentucky. Mr. Rust is also superintendent of the Dairy Center (the U. K. dairy cattle unit).

Oregon to Hold Annual Meeting

The 44th annual Dairy Industries Short Course and convention will be held at Oregon State College, Corvallis, Feb. 14-17, 1955. Several top-flight men from different parts of the United States will appear on the program. H. B. HENDERSON, head of the Dairy Department, Univ. of Georgia, will be one of the visiting instructors. The last day will be devoted to discussions on merchandising and selling dairy products. The main speaker at the annual banquet of the Oregon Dairy Industries Assoc. on Feb. 17, will be RICHARD WERNER, executive director, Milk Industry Foundation, Washington, D. C.

Turk in Philippines

K. L. TURK, head of the Animal Husbandry Department at Cornell Univ., left Ithaca Sept. 13 for a year's work at the College of Agriculture of the Univ. of the Philippines at Los Banos. Mrs. Turk accompanied him.

The Cornell scientist will continue the studies in animal nutrition, breeding, and other livestock research begun by J. K. LOOSLI, who is returning to the Department of Animal Husbandry after a year at Los Banos. This research is intended to increase and improve the quality of livestock in the Philippines. Professor Turk will assist also in the development of the teaching program and with other staff members promote closer relations between the College and industries in the Philippines in an effort to obtain financial aid for agricultural research.

The first contract between Cornell Univ. and the Univ. of the Philippines was signed in 1952 and has been renewed this year for three more years. Under this contract a staff of specialists, mainly from Cornell, is sent to the College of Agriculture at Los Banos. The program is financed largely by the Foreign Operations Administration, supplemented by funds from the Philippine government. Under the new contract, the staff of American specialists has been increased from 10 to 14.

W. H. E. Reid Returns from European Trip

W. H. E. REID of the Univ. of Missouri has returned from a 6-week trip to Europe, where he studied dairy operations. He was accompanied on the trip by Mrs. Reid. Professor Reid reports that most of the countries visited were unusually cool and rainy this summer.

Coulters to Take Trip Around the World

DR. and MRS. S. T. COULTER sailed Sept. 25 for France on a trip that will take them around the world. On Oct. 10-18 Dr. Coulter will participate in a seminar on "Milk in Warm Countries," to be held at Amalfi, Italy. This seminar is sponsored jointly by the Intern. Dairy Federation and the Food and Agriculture Organization of the United Nations.

In addition, Dr. Coulter will attend the training center on Milk Production Processing and Distribution to be held at the Aarey Milk Colony, Bombay, India, from Oct. 24 to Nov. 20. This meeting is sponsored by the Food and Agriculture Organization of the United Nations. The Coulters plan to complete their trip around the world from Bombay.

Rhode Island News

G. K. WILDES, Univ. of Rhode Island graduate, was employed as instructor and superintendent of the Univ. Dairy Farm, July 1, 1954. He replaced FREDERICK WARREN, who accepted a position with the Hoffman-LaRoche Chemical Co. of New Jersey as a research farm manager.

R. M. PARRY, D.V.M., was employed as lecturer in dairy products work. Dr. Parry is at present directing graduate studies in dairy products chiefly concerned with quality milk control.

JOHN VERNA received his Master's degree in June, 1954. His thesis work was chiefly concerned with the bacteriological phases involved in the study of granular vaginitis in the bovine animal. Mr. Verna is now enrolled at Brown Univ. working on his Ph.D. degree with part-time employment at Chapin's Hospital in Providence.

M. H. CAMPBELL, dean of the College of Agriculture, who is serving as Chief Agriculturist in Egypt, expects to return to the University in February, 1955.

Recent Personnel Changes at Minnesota

HOWARD THOELE, former research fellow, will join the Pennsylvania State Univ. Extension Staff (Dairy Cattle Breeding) about Nov. 1.

RICHARD ADAMS will assume his duties on Nov. 1 as animal nutritionist on the Pennsylvania State Univ. Extension Staff.

GERHARD HARPESTAD left Sept. 1 to join the Extension Staff in Dairy Husbandry at the Univ. of Illinois.

Eldridge Joins Nebraska Staff

F. E. ELDRIDGE has resigned his position as a member of the Kansas State College Department of Dairy Husbandry to accept an appointment as associate director of resident instruction in the School of Agriculture at the Univ. of Nebraska. Dr. Eldridge has been in charge of instruction and research in dairy cattle breeding at Kansas State for the past 7 years.

Harmon Goes to M.S.C.

L. G. HARMON, formerly on the dairy manufactures staff at Texas Technological College, has joined the dairy staff at Michigan State College, where he will develop a marketing research program on dairy products.

Curriculum Changes at M.S.C.

In 1951, the Michigan State College dairy department graduated only three students who had majored in dairy production. A review of student enrollment indicated no improvement in the situation could be expected during the next 3 years.

Following consultation with students, graduates, and staff, it appeared that the major criticism of the then existing curriculum was that it did not provide a proper balance between dairy cattle nutrition, breeding, and management. This condition was rectified and during the 3 years operation of the present plan, the average number of graduating students has risen to seven each year. Further increases are expected during the next 3 years. These numbers compare very favorably with the 1930-1939 average of 7.4 graduates per year and the 1940-1949 average of 7.3. However, in view of the increased all-college enrollment since 1945, the number of dairy production graduates is still alarmingly low.

Further modernization of the dairy production undergraduate program to incorporate a business option is contemplated as a means of encouraging student enrollment. This program would be similar to the administration and sales option which was adopted in 1953 for students majoring in dairy manufactures.

Students in dairy manufactures must complete 192 quarter credits for graduation, 120 of which are from required subjects in the arts and sciences, including agricultural science. Thus, 72 elective credits remain in general business, economics, accounting, and journalism for students who wish to specialize in administration and sales. Students interested in dairy plant production, control, or quality work may take their electives in chemistry, bacteriology, and applied science.

Michigan to Hold Annual Dairy Manufacturers Conference

The 15th annual Michigan Dairy Manufacturers Conference will be held at Michigan State College on Nov. 3 and 4. Local and nationally prominent dairymen will be featured in sectional programs on market milk, ice cream, butter, and manufactured milk products. A products clinic, inaugurated at the 1953 conference, will be continued with strawberry ice cream, cottage cheese, and buttermilk to be critically judged.

One of Michigan's outstanding dairy leaders will be presented with the second annual Michigan State College Dairy Manufacturers Award at the banquet to be held the second night. At the conclusion of the conference, J. M. JENSEN of the Michigan State staff will conduct a 1-day clinic on cottage cheese making.

Completed Theses

M.S. Degree:

CECILIA DEMBICZAK—Three-day versus one-day growth measurements of the young dairy calf. Univ. of Connecticut.

JAMES WALKER-LOVE—Observations on self-feeding roughages to dairy cows in loose-housing. Michigan State College.

MARLOWE E. NELSON—A comparison of methods of evaluating butterfat production for developing a dairy breeding program. Michigan State College.

L. A. RICHARDSON—Water-insoluble fatty acid content of farm separated cream in relation to certain other quality characteristics. Univ. of Kentucky.

T. W. DENTON—The effects of an Aureomycin supplement and certain surface active agents on apparent digestibility and blood levels of urea nitrogen and total non-protein nitrogen in young dairy calves. Univ. of Kentucky.

JACKSON A. TAYLOR—A comparison of various grasses as to composition, digestibility, and forage consumption under grazing conditions. Univ. of Kentucky.

Ph.D. Degree:

HAROLD W. JACKSON—Identity and origin of the malty aroma substance from milk cultures of *Streptococcus lactis* var. *maltigenes*. Univ. of Connecticut.

LEONARD R. MATTICK—Quantitative determination of antibiotics in milk. Univ. of Connecticut.

EILER S. HUMBER—The role of added nonfat dry milk solids and the pressure of homogenization on the stability, viscosity and other properties of half-and-half homogenized milk. Michigan State College.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

TRENDS IN PACKAGING OF FOOD

MARIAN G. KLEIN
Marathon Corporation, Menasha, Wisconsin

A very curious thing has taken place in this country—and almost without our knowing about it. A revolution is under way in the food market, and the food industry appears only vaguely aware of it. Back in 1941, Americans spent $20 billion for food. In 1953, they spent $60 billion. Over the past 12 years, the increase in U. S. food expenditures has been greater, dollarwise, than the increases in spending for homes, consumer durables, or automobiles—or even for all of these combined. Briefly, the American people decided that food was more important to them. In 1941, they were allocating 22% of their cash income for food. In 1953, the figure was up to 26%.

Over the past decade and particularly since 1946, the American public has made a radical change in its habits of buying food. Some of the factors responsible for this change are:

1. *Mechanization of packaging.* The development of automatic fast-moving packaging lines made possible mass volume production of packaged foods. With volume and variety in packaged foods came the evolution of the self-service stores.

2. *Growth of supermarkets and self-service.* Supermarkets now do over half of the U. S. grocery business. The first impression upon entering a well planned self-service store is an impression of tremendous quantity and variety of merchandise, most of it in colorful, attractive packages. With the wide assortment of neat, attractive food packages on the carefully arranged display shelves, the customer is greatly tempted and usually ends up buying a greater quantity of merchandise than she had planned to buy. We are aware of the tremendous amount of "impulse buying" which goes on in the modern self-service stores. Surveys have indicated that when husband and wife shop together as a team, there is more "impulse buying" than when either person shops alone.

While the art of store arrangement, display, and good packaging has encouraged impulse buying to the benefit of the store, it has also helped the shopper to buy her food and household needs quickly, pleasantly, and economically.

3. *Population movements.* More and more of our population is moving to industrial areas and living in the suburbs of large cities. The tendency of industry to decentralize, the fear of the atomic bomb, traffic congestion, and the development of housing units have caused a flow of population away from the larger cities into smaller centers. In addition to the above change, our population is moving westward and south-eastward at the expense of the middle west and northeast. These migrations mean great changes in living standards. New wants, preferences, taste, and viewpoints resulting from migration greatly affect purchases.

4. *Growth of outlying shopping centers.* The widespread decentralization movement of population and industry has encouraged the rapid growth of neighborhood stores and secondary shopping centers.

Shoppers made it clear to the grocers some years back that they were determined to shop less frequently for food. And the automobile made it possible to carry off the necessarily larger purchases. So by forceful persuasion, the food stores began to provide customers with parking space. But the provision for space meant that the stores could not be located in the older downtown shopping districts or in the densely populated residential areas. Consequently, shopping centers in outlying areas sprang up. At these centers once-a-week shopping is the rule instead of daily shopping trips, as was formerly the case. The trend toward larger home refrigerators and refrigerator-freezer combinations allows the shopper to keep more food in her home for longer periods of time.

5. *Expanding middle class with incomes of $4,000 to $7,500.* After 1947, consumers with family incomes of $4,000-$7,500 per year began to dominate the food market. These groups can afford to pay for considerable processing and other services in their food purchases. They buy "convenience" foods, i.e., foods with built-in service. On the other hand, people in the lower income brackets buy basic foods and do much of the work required in preparation of their food products. Surveys have indicated that people in the higher income brackets do not buy more food, as measured in pounds, but they do buy higher-priced foods.

6. *Larger number of married women in business.* Today, 30% of the homemakers are working in business. This fact, together with the shortage and high cost of domestic help, means that these housewives have very little time for

preparation of food in the kitchen. Consequently, they demand and buy convenience type foods which can be prepared and served in a hurry.

7. *Increase of convenience food items.* This great demand for convenience built into foods has been met by the industry. Today on the market there are all types of prepared mixes (cake, biscuit, pancake, frosting, frozen dessert, candy, etc.) which are easy to use and quick to prepare. It is estimated that over 20% of the fresh meat sold in the U. S. has been trimmed, prepackaged, and labeled. We also see frozen fruit juices, frozen vegetables which are ready to drop into the pot for boiling, frozen precooked individual items which require only heating, and now even a frozen dinner, complete with meat, gravy, potatoes, and vegetables—all ready to heat and serve.

8. *Television.* Television in the homes also has tended to remove the cooks from the kitchen. And again the American housewife insisted on foods which are easy and fast to prepare so that no important program would be missed.

9. *Diet changes.* With development of machinery to do most of the heavy work, heavy labor has been reduced, and the need for high calorie diets also has been reduced. Consequently, in recent years we have seen a swing away from the high calorie carbohydrate diets and toward the lower caloric, protein type diets.

10. *Advertising.* All forms of advertising (television, radio, newspaper, and magazines) have been very effective in getting people to try new products and to shift from one brand to another. Advertising is a potent force in creating and changing consumer habits and wants.

Because of the self-service boom, purchasers have become accustomed to choosing the items they buy, and they are becoming more critical of the merchandise. As a result of this critical viewpoint, the selection of the packaging materials has become more important. In selection of a package and its component materials for a given product, four primary points must be considered:

1. *Sales appeal.* Since the package must stimulate sales and encourage repeat sales, it must have genuine visual appeal. Color is perhaps the most vital factor in commanding attention. After the package has caught the eye, it must identify the product and inform the customer. Here typography and design are important. Finally, the package, by pleasing the eye and the emotions, must invite the consumer to purchase the product.

2. *Protection.* The first duty of the package is to protect its contents. If a package fails to do this, there may be no first sale of a product, and there surely will be no repeat buying. Good packaging materials can protect product quality against the following hazards:

a. Moisture loss or gain as influenced by external humidity conditions
b. Damage by exposure to atmospheric oxygen
c. Mold and bacterial action
d. Loss of flavor
e. Adsorption of foreign odors
f. Sifting of powders and leakage of juice
g. Light penetration
h. Chemical reaction between product and container
i. Seepage and staining of fat or oil

There is no all-purpose container which can be used for packaging all foods. Each container must be tailor-made to fit the specific characteristics of the individual food product. After determining the product requirements, the packager must make a sound choice of available packaging materials and adopt these materials to requirements of high speed filling, closing, and handling operations.

3. *Convenience.* The great demand for convenience has led to improvements in packaging. In designing packages which will move in self service markets, the manufacturer cannot afford to overlook factors relating to convenience and utility of the package in actual use. Easy opening and reclosure features, as tear tapes, metal pour spouts, and pitcher pour spouts on paper milk bottles, are aids which facilitate use of the package. The convenience of accurate, premeasured portions in the portion-controlled containers is appealing because they eliminate less accurate spoon-measuring from jars and cartons. Another package convenience which has proven popular is the carry-home carton for glass bottles and metal cans.

If plus values can be built into the package which make it easier for the buyer to use, repeat sales are insured.

4. *Economy.* The package must be economical or it never will attain volume sales. Choosing component materials which are abundant in supply and which will permit high speed fabrication will help to keep costs at a satisfactory level.

If the package does not have the proper balance in respect to these four points—sales appeal, protection, convenience, and economy—its promotion and outlook for a continued market are hampered.

Packaging Trends

In the large supermarkets today even the humblest package must take on the duty of salesmanship. There is a trend toward brighter and bolder colors on packages, probably influenced by the number of men shoppers these days. The primary colors, red, blue, and yellow, are becoming very prominent on food packages. As for design, the pictorial design is still very popular. However, the trend is toward showing a colorful illustration of the food product in end use rather than a picture of the product

itself. In the dairy field, the picture of the cow and the pasture is giving way to appetite appealing designs. The designs which were created for the American Dairy Association for its big promotion to sell butter will show a stack of light brown, steaming pancakes with several pats of butter on top—or a brown, roasted turkey dripping with butter—rather than a picture of butter itself. Television, too, has played an important role in influencing packaging design. It has started a trend toward simplification of the all-over package design. Neater, clean-cut designs are the result of this trend.

Changes are also being made to insure package visibility and to aid in identification of packages at a distance. Since a portion of the shoppers move through food stores in a hurry and since not all packages can be displayed at eye level, many small packages with poor visibility are overlooked. To attract the shopper's eye, some packages are now being designed with the illustration and brand name on the top, sides, and bottom panels rather than only on the top panel. This gives the package multisided visibility which insures that the package will win attention regardless of its position in the store display. Concise, informative labeling and copy large enough to read at a glance are aiding package legibility. The importance of legibility was brought out clearly in recent studies on buying habits conducted by Marathon. It was learned that many packages were not dropping into the shopping baskets because the shoppers had left their glasses at home and could not read the finely printed words on the packages. Since the package should serve as a billboard, emphasis is being placed on shape and size, color combinations, selection of typography, and brevity of sales points. In addition, useful information, such as recipes and serving suggestions, is appearing on the new packages to help the customer in her use of the product.

As for packaging materials, the greatest advances are being found in the field of new films and highly protective laminations. These sheet materials are finding increasing use in single-service packages. New coatings and laminations are giving them greater strength, excellent heat seals, improved protective qualities, and increased adaptability to high speed equipment.

Much use is being made of aluminum foil. The improvements in processing lighter weight aluminum foil are creating fields of packaging that formerly were considered impossible. The lighter weight foils are not being used singly, but in combination with other sheet materials for two-ply and three-ply laminations.

Very versatile high speed machinery has been developed to handle these highly protective films and laminations. Most of the new pouch-forming machines can operate at a rate of 120 to 300 packages a minute. These machines are available for automatic forming, filling, and heat sealing; some include printing, coding, and perforating attachments. Vacuum packaging machines with six and eight head rotary units are capable of pulling a vacuum and heat-sealing pouches at the rate of 40 packages a minute. Because of the lack of a good, approved antimycotic, vacuum packaging is becoming more popular, especially for sliced cheese (both process and natural) and for sliced luncheon meat in order to protect product quality for prolonged periods. And in the ice cream field the development of a new, sliced brick machine has modernized the method of handling this product. In addition to versatility, the new packaging machines require less maintenance and can be operated by less highly skilled personnel.

In the carton field, containers of all types are being made to finer tolerances for efficient use on high speed automatic packaging lines. The carton manufacturers are combining grease-proof papers and films with paperboard, and they also are applying heavier wax coatings to improve greaseproofness and moisture protective properties.

The average supermarket today carries approximately 5,000 items, which are packaged in many different types of containers. Some of the various package forms are shown in the accompanying photographs.

FIG. 1. One-portion packets

In Figure 1 a number of one-portion packets are shown. These packets usually contain a premeasured portion of product, enough for a single service. As shown in the photograph, some powdered milk is now being packed in a moisture-proof paper-foil laminate which is polyethylene coated on the inner surface (foil side) for heat sealing. Portion-controlled amounts of dehydrated cream product can be purchased in a triple-ply laminate of acetate-foil-pliofilm. A triple-ply sheet of foil-paper-foil is now being employed to pack frozen dessert mix powders. When aluminum foil is exposed on the outer surface of the pouch, as in the dessert mix package, some precautions must be taken to protect the foil from mechanical and physical damage. This can be accomplished by varnishing or lacquer-coating the foil or by using heavier gauges of foil.

A 10½-oz. overwrapped carton containing a granular nonfat milk product was recently

FIG. 2. Unit packaging.

introduced. This carton has a convenient metal pour spout for easy removal of the product. The highly protective overwrap is fabricated of a cellophane-foil lamination with a heavy wax-type coating on the foil side for heat sealing.

Unit packaging is illustrated in Figure 2 by a photograph of half-pound packages of sliced process and natural cheese, link cheese, sliced luncheon meat, cartoned sausages, and individual 4-oz. ice cream packages. The sliced natural Swiss and sliced luncheon meat have been vacuum packed for longer keeping.

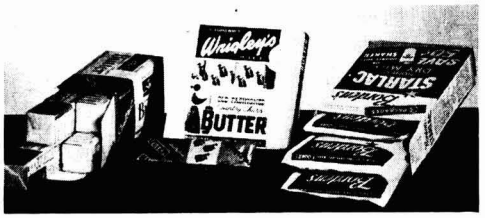

FIG. 3. Fractional packaging.

Another type of packaging known as "fractional packaging" is shown in Figure 3. It features a standard put-up divided into small protected units. In this photograph, quarter-pound prints of butter are packaged in waxed paper and also in laminated foil. Research is under way at the present time on a reverse foil butter wrap. The individual powdered milk pouches are fabricated of a paper-foil laminate which is coated with polyethylene on the foil side. Fractional packaging is popular because the units are protected up to the point of actual use. This enables the shopper to stock larger quantities, keep them fresh longer, and eliminate extra store trips.

Just the opposite of fractional packaging is "multiple packaging." As shown in Figure 4, a number of small wrapped units have been banded or cartoned to attract bargain seeking customers and to prevent pilferage.

Economy minded customers will be interested in the "king size" packages. In Figure 5 are shown 2½-lb. packages of frozen vegetables, 5-lb. packages of frozen shrimp, and ½-gal. packages of ice cream, which are prominent in the reach-in cabinets.

Overwrapped dairy packages are showing up occasionally. Pint and ½-gal. cartons can be found overwrapped with a single sheet of heavily waxed paper. These are not insulated packages. The overwrap is applied directly to the carton to provide better long-term keeping qualities. The foil overwrap on the butter package, used in conjunction with parchment wrapped prints, provides glamor and extra protection.

FIG. 4. Multiple packaging.

Modernization of an old idea is illustrated in Figure 6. Here, in the Arden and Westwood designs, is a modernized version of the old half-gallon pail. The shape of the old pail has been retained, but the new pail is shipped flat with an automatic bottom for easy set-up by hand or by automatic machinery. As can be observed in the photograph, the top lock flaps on the new pail have been altered slightly for automatic machinery.

The TV frozen dinner is an example of ultra convenience. This complete meal, consisting of meat, dressing, potatoes with a pat of butter, and vegetables with a pat of butter, comes packaged in an aluminum tray and is ready to be placed into a hot oven. After heating, the meal can be served in the aluminum tray, and upon completion of the meal the tray can be thrown away, thus eliminating dish washing. This package is protected by a new cellophane-tissue laminated overwrap. The new overwrap has high gloss, good heat seals, and

FIG. 5. King-size packages.

good strength at temperatures of 0° F. to –20° F., whereas cellophane alone becomes very brittle at low temperatures and has a tendency to shatter.

All of the packages illustrated in the various photographs have shown a rapid gain in consumer acceptance—principally because of the proper balance in economy, quality protection, convenience, and attractiveness.

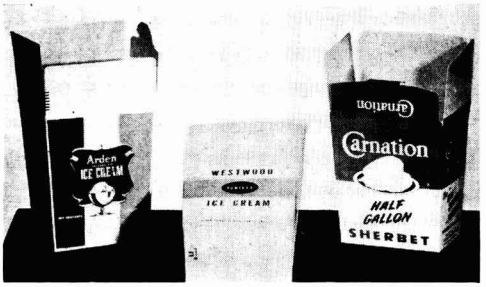

FIG. 6. Modernized version of half-gallon ice cream pail.

In addition to the trends toward new designs, new package materials, new machinery, and new package forms, there also is a trend toward more research and better organized programs to develop new uses, new markets, better methods and new equipment. This increased research is being stimulated by competitive pressure. Already there are better coatings, linings, and films which improve the physical and protective properties of packages, in many cases at reduced cost. More packaging operations are becoming mechanized, and equipment to run at greater speeds is continually being developed.

The buyer's market is encouraging better packaging. As a result, consumers will benefit because at no increase in cost they will get foods in packages that are more convenient, reduce spoilage, and avoid waste.

THE INFLUENCE OF DAIRYMEN'S KNOWLEDGE ON THEIR SUCCESS AND ACTIONS[1]

C. V. HESS AND L. F. MILLER

Department of Agricultural Economics
Pennsylvania State University

Many hypotheses of an economic, social, and personal nature have been advanced to explain why some dairymen have failed to improve the efficiency of their dairy operations. One of several such hypotheses tested in a study in central Pennsylvania was that some dairymen lacked the necessary knowledge of improved production practices and principles.

The 151 dairymen used in this study represented a sample of the owner-operated dairy farms[2] located on limestone soils. Although approximately one-third of the farmers in this area are tenants, only owner-operators were contacted for this study because of a general tendency for tenants to rationalize many of their decisions and actions in light of their existing tenure agreement.

The first step in appraising the role of knowledge in relation to dairymen's methods was to develop a representative list of test questions in cooperation with dairy extension specialists at the Pennsylvania State University. The questions asked each of 151 dairymen, and their answers, are given in Table 1. Scores to be assigned each answer were determined with the aid of the dairy specialists. Although this list of questions is short, it is felt that the answers provided a reasonably valid index of the farmers' knowledge of improved dairy management.

Individual operator scores ranged from 96 to 10 with a mean score of 66 (perfect score of 100) and a standard deviation of 18 (Table 1). This range in scores suggests that the test did segregate dairymen into different knowledge groups on the assumption that the operators were examined in the significant areas of knowledge dealing with the dairy enterprise. It is interesting that the average scores were fairly high on questions 3, 5, and 7 dealing with the relative protein and TDN value of different feeds and with the principle of diminishing returns. In contrast, average scores were low on the remaining questions dealing with the amount of concentrates to feed, the proper protein level, and selection of the herd sire. In these areas there appeared to be a serious lack of knowledge on the part of many dairymen.

Dairy Knowledge and Success in Farming

The apparent relationship of knowledge to the success of the total farm business, and more specifically the dairy enterprise, was examined by relating the scores made by these operators to such factors as labor income, returns above feed costs per cow, and milk production per cow (Table 2). The level of farmers' knowledge, at least with regard to the dairy enter-

[1] Authorized for publication on *March 25, 1954* as Paper No. *1867* in the journal series of the Pennsylvania Agricultural Experiment Station.

[2] A dairy farm was designated as one on which six or more cows were maintained and from which fluid milk had been sold for at least 9 months of the year studied. A comparison of certain characteristics of the owner operators used in this study with similar data for approximately 80% of all owner dairymen in the area revealed no appreciable differences with respect to such factors as the age of the operator, size of herd, and production per cow. However, the level of formal education of the farmers included in this study was significantly higher than for all owners, with 46% of the operators in this study having completed more than eight grades, compared with only 36% for all owners. The difference was significant at less than the 0.05 level by Chi square test.

prise, appears to be closely related to the degree of success not only of the dairy enterprise but also of the total farm business. The operators with higher knowledge scores were operating farms with substantially larger total capital investments.

The apparent influence of knowledge on returns was most evident at the higher levels of capital investment. For example, for an average capital investment of $14,000, there was an associated increase in labor income of $9.12 for each point increase in knowledge score. In contrast, the associated responses in labor incomes per unit increase in knowledge score for average investments of $21,000 and $35,000 were $18.17 and $36.27,[3] respectively. Therefore, improved knowledge scores were associated with four times the response in labor incomes at the higher capital investment level than at the lower investment level. However, since improved knowledge is related to a number of other desirable characteristics of operators, it would be unfair to attribute all of the associated response in labor income at higher investment levels to improved knowledge alone.

TABLE 1

List of questions asked 151 owner-operator dairymen; mean test score and standard deviation (central Pennsylvania, 1949)

Question	Perfect score	Mean score	Standard deviation[a]
1. What is the recommended rate of feeding grain to Holsteins and Guernseys?	15	9	6
2. In your mind what should be considered in deciding upon the rate of feeding grain to dairy cows?	20	9	4
3. Rate the following grains and concentrates in order of greatest to least feeding value (protein basis) for dairy cows: corn and cob meal; wheat bran; soybean oil meal; oats.	15	13	3
4. What percent crude protein should your dairy ration be with liberal feeding of the following roughages? legume hay (properly cured) with corn silage; good mixed hay with corn silage or good mixed hay alone; nonlegume hay with or without silage.	15	8	5
5. Rank the following from highest to lowest feeding value per acre under average weather conditions and with these assumed yields: corn silage (10 tons/acre); oats (40 bu/acre); clover hay (2 tons/acre); good pasture (permanent or semi-permanent).	10	8	2
6. Rank in order of importance the following considerations in choosing a bull calf for a future herd sire: milk production record of the dam; milk production record of his full sisters and half sisters; presence of world champion milk records in the blood line about three generations back.	15	10	3
7. An average herd receiving the usual amounts of grain and roughage was fed an additional 300 lb. of grain which resulted in an additional 400 lb. of milk. If another 300 lb. of grain is fed, what effect on milk production would you expect? About another 400 lb. increase in milk production; something less than a 400 lb. increase; something more than a 400 lb. increase.	10	9	3
Total	100	66	18

[a] Standard deviation is a statistical measurement of the degree of dispersion of scores around the mean score of 66. The mean score (66) plus or minus the standard deviation (range of scores from 48 to 84) would include scores made by approximately 68% of the 151 operators, based on a normally distributed frequency of scores.

Analysis disclosed a high degree of relationship between the level of formal schooling and the scores made on the knowledge test. In view of this an effort was made to test the importance of knowledge within each of the formal schooling groups (Table 3). Within the grade school group of farmers, the 33 operators who made scores of over 65 on the knowledge test had significantly higher labor incomes and higher production per cow and were operating larger dairies with nearly $7,000 higher investment. Also, they were a younger group of farmers. The same general relationships ex-

[3] Based on least squares evaluation of hypothetical relationship where: Labor Income = a + b (capital investment) + c (knowledge score) + d (product of capital investment and knowledge score.)

TABLE 2

Relationship of knowledge to the success of the farm business and the dairy enterprise (151 central Pennsylvania dairy farmers, 1948-1949)

Ranking of scores	Number of cases	Average score	Pounds milk per cow[a]	Productive man work units[a]	Average capital investment[a]	Labor income[b]
Lower 3/10	45	43.9	7,373	428	$18,928	$1,852
Middle 4/10	62	67.8	7,994	460	23,104	2,805
Upper 3/10	44	85.6	8,241	566	27,879	3,873
Average	151	65.8	7,881	481	$23,251	$2,832

[a] Mean differences are significant at less than 5% level by analysis of variance test.

[b] Labor income represents the annual net cash farm income (adjusted for inventory changes) minus a 5% interest charge on the capital investment and the estimated value of unpaid family labor.

isted within the two other schooling groups. There were only 18 operators with more than a high school education. When these operators were grouped into the "over 65" and "65 or less" knowledge score groups, only three were in the low knowledge group, so that little can be said concerning the relationships found here.

When the average labor incomes for each of the formal schooling groups were adjusted to the average knowledge score (influence of knowledge score on labor income is thus removed) the adjusted average labor income for the grade school operators was $2,547, as compared to labor incomes of $3,166 and $3,286 for the high school and more than high school groups, respectively. These data would suggest that the level of formal schooling or the personal characteristics generally associated with more formal education are also important contributing factors to success in farming. However, it does not imply that grade school operators are doomed to failure, because the data disclose that acquired knowledge, as measured partly by the knowledge test, is a more important determinant of farming success than the level of formal schooling attained.

Knowledge Related to Personal Characteristics and Actions

The mere possession of knowledge in itself is no assurance of financial success. This poses the question as to what other characteristics or traits are associated with the operators who have acquired this farming "know how." Can we "type" the high- and low-knowledge groups of operators?

The high-knowledge group (the upper 30% of the operators as arrayed on knowledge test scores) as compared with the low-knowledge group (the lower 30%) took a more active part in community organizational activities and more of them belong to the local Dairy Herd Improvement Association. The high-knowledge group also had a better idea of what was considered a "good" rate of milk production per cow, had a much higher standard of satisfactory performance as indicated by a high level of production below which they cull cows from their herd, and finally tended to rate themselves above their neighboring dairymen with respect to their production per cow and as all-round farmers. High knowledge scores are thus associated with other characteristics which are usually considered desirable.

TABLE 3

Relationship of knowledge within formal schooling groups to several personal and business factors (147 central Pennsylvania dairymen, 1948-1949)

Level of schooling	Average knowledge score	Knowledge score groups	Number of cases	Average score	Labor income[a]	Unadjusted labor incomes	Adjusted labor incomes[b]
Grade school	62	65 or less	46	51	$1,928	$2,414	$2,547
		over 65	33	78	3,089		
High school	68	65 or less	18	45	2,860	3,231	3,166
		over 65	32	80	3,440		
More than high school	77	65 or less	3	56	2,739	3,697	3,286
		over 65	15	82	3,888		
Average			147	66	$2,848	$2,848	

[a] Analysis of covariance shows mean difference adjusted to the mean knowledge score groups to be significant at less than the one per cent level.

[b] Labor incomes adjusted to the mean knowledge score.

Since knowledge provides the basis for correct decisions and actions, what is the relationship between the level of knowledge and actions by farmers? Do those who possess the knowledge actually apply it in their decisions and actions? Evidence to support the hypothesis that correct dairy knowledge usually promotes what is generally considered desirable actions is represented in Table 5. The high-knowledge group as compared with the low-knowledge group changed the protein level of their dairy grain ration more often during the year in response to changes in quality of their roughages. Of the 39 operators who were feeding hay and/or silage as supplemental feed during part of the summer period because of a pasture shortage, 30 indicated they had taken measures over the past few years to correct this condition. Forty per cent of these operators were in the upper-knowledge group and only 17% were in the low-knowledge group.

When asked whether their milk production has changed during the last 3 or 4 years, a higher percentage of those indicating an increase was in the high-knowledge group. Most of those indicating no change were in the low-knowledge group. Of the operators anticipating an increase in production over the next 3 or 4 years, a significantly higher percentage was in the high-knowledge group, and most of those expecting no change in production were in the low-knowledge group.

When asked whether they had thought seriously about any changes to reduce the time required to do dairy chores, of those answering "yes," 41% were in the high-knowledge group with only 23% in the low-knowledge group. This would seem to indicate greater concern and foresight on the part of the high-knowledge group with regard to future efficiencies in the operation of the dairy.

Summary

The results of a knowledge test, which quizzed 151 central Pennsylvania dairymen over selected dairy management practices, suggest that a high proportion of our dairymen lack much of the basic knowledge required for proper decision making. Scores on the test ranged from 96 down to 10 with an average score of

TABLE 4
Relationships of knowledge to certain characteristics of operators (151 central Pennsylvania dairymen, 1948-1949)

Characteristics[a]		Number of cases[b]	Percentage of operators in different knowledge score groups	
			Upper 3 deciles	Lower 3 deciles
Progressiveness:				
What year did you adopt hybrid corn?	1941 or earlier	62	39	16
	Since 1941	80	22	38
Do you take part in organizations?	Yes	72	40	14
	No	79	19	44
Do you belong to DHIA?	Yes	32	62	0
	No	115	21	36
Concepts and standards:				
What do you consider to be a good rate of milk production per cow?	11,000 or more[c]	28	50	14
	9,500-10,999	22	41	4
	Less than 9,500	31	42	13
	Doesn't know	67	10	51
Down to what level must a cow drop before culling?	8,000-10,000 lb.	16	56	0
	6,000-7,999 lb.	31	45	7
	Under 6,000 lb.	20	30	25
	Doesn't know	83	17	46
Farmer's self-rating:				
As all-round farmer	Upper 1/5	53	45	23
	Second 1/5	26	23	23
	Lower 3/5	68	18	36
On milk produced per cow	Upper 1/5	44	48	18
	Second 1/5	29	24	21
	Lower 3/5	71	22	39

[a] All relationships significant at 5% level or less by the chi square test.
[b] The number of cases varies quite often from the total of 151 farmers in the study because some operators were not able to answer all the questions. In other instances only the important answer categories are indicated on the table.
[c] Productions given by farmers have been corrected to 4% butterfat basis.

TABLE 5

Relationship of knowledge to certain actions of operators (151 central Pennsylvania dairymen, 1948-1949)

Actions of operators		Number of cases	Percentage of operators in different knowledge score groups	
			Upper 3 deciles	Lower 3 deciles
Do you change protein level of grain ration during the year?[a]	Yes	102	31	23
	No	49	24	45
Does quality of your roughage influence protein content of your grain ration?	Yes	108	34	22
	No	42	17	50
Have you taken any measures during last 4 or 5 years to correct the pasture shortage?[a]	Yes	30	40	17
	No	9	22	56
Has milk production changed any in last 3 or 4 years?	Yes, increased	97	32	21
	No change	38	24	52
What change in milk production do you expect in next 3 to 4 years?	Increase	120	32	23
	No change	26	15	50
Hhave you thought about any changes to reduce time to do dairy chores?	Yes	58	41	23
	No	55	15	38
Why have you never joined a DHIA?	Cost too great	26	15	31
	Never bothered	40	12	50
	Miscellaneous	36	28	25

[a] Relationship significant at 10-20% level by the chi square test. All other relationships are significant at 5% level or less.

66 and a standard deviation of 18. The gap between the present state of knowledge of many dairymen and that required to actually adopt improved practices is apparently greater than frequently appreciated. The importance of overcoming knowledge deficiencies is suggested by the fact that the 44 operators who made high scores on the knowledge test had herds averaging nearly 1,000 lb. higher production per cow, with $40 higher returns above feed costs per cow, and were operating farms earning labor incomes averaging $2,000 over those farms operated by the 45 operators who made lower scores on the same knowledge test.

Knowledge is particularly important for dairymen operating with high capital investment. The associated response in labor incomes to improved knowledge was four times as great on farms operating with a high capital investment as on farms operating with a low capital investment. Likewise, the operators making the higher scores possessed many other characteristics or traits usually considered desirable, had adopted more of the recommended dairy management practices, were anticipating future increases in milk production, and were seriously concerned about taking measures to further improve their dairy operations.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

903. Control of mastitis. L. K. WAYT, School of Vet. Med., Colo. A. & M. Coll., Fort Collins. J. Milk and Food Technol., **17**, 8: 243. 1954.

A cooperative effort should be made to properly inform the dairyman of the symptoms, means of detecting mastitis in the cow, and the interpretation of the laboratory examination of the milk samples. If this procedure was followed the incidence of mastitis in the dairy herd could be greatly reduced. H. H. Weiser

904. New studies on comparative Brucella immunity with agglutinogenic and nonagglutinogenic vaccines. F. SIEIRO, Inst. Rosenbusch, S. A., Buenos Aires, Argentina. Am. J. Vet. Research, **15**, 56: 417. 1954.

Three groups of heifers were used as (1) control, (2) vaccinated with BAI strain 19, and (3) vaccinated with Rosenbusch nonagglutinogenic strain B. At 5 to 7 mo. gestation the heifers were inoculated with a virulent strain of *Brucella abortus* at two levels. Most of the control group aborted. The difference between strain 19 and strain B was slight—50 and 47.6% respectively did not abort from the lower dose. It is suggested that strain 19 produced a slightly higher immunity. For this reason it is proposed that a control program could use both vaccines by vaccinating calves with strain 19 and using strain B for revaccination of heifers or for vaccinating adult animals. In this manner the agglutination test could still be used satisfactorily in the control proram.

E. W. Swanson

905. The teeth of the ox in clinical diagnosis. II. Gross anatomy and physiology. N. L. GARLICK, Tacoma, Wash. Am. J. Vet. Research, **15**, 56: 385. 1954.

Detailed descriptions of the variations of normal teeth in cattle are presented along with excellent illustrations. Dairy and beef breeds differ in the wear and attrition of the permanent teeth, with teeth of the dairy cattle remaining longer and in stronger condition. Tables of changes with age in deciduous and permanent teeth are given for beef and dairy cattle. It was noted that loss of teeth was more dependent upon age than upon the damage to the teeth. Gingivitis at the time of dental eruption is a normal occurrence which should not be confused with disease processes.

E. W. Swanson

BOOK REVIEWS

906. Livestock Production. WALTER H. PETERS and ROBERT H. GRUMMER. 2nd edition. McGraw-Hill Book Company, Inc., New York. 415 pp, 101 illustrations. 1954. $6.00.

This text is designed for use in a survey course for beginning college students in agriculture. The book covers in brief the production fields in beef cattle, dairy cattle, swine, sheep, goats, horses, and mules. Feeding, judging, marketing, health, breeds and breeding are discussed for each class of livestock. The book is interestingly written, but quite brief in its coverage of each topic.

Very little attention is given to the more modern methods of dairy management. It is apparent that the book will be of limited value where any number of the beginning students in a course have had previous contact with the dairy field.

It remains questionable whether the text is of college level, although it could prove informative, particularly to students of limited agricultural experience. K. E. Gardner

BUTTER

907. A device for rapidly churning small quantities of cream. R. M. DOLBY, Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **21**, 1: 78. 1954.

A device for churning small quantities (200-500 ml.) of cream is described. The cream is churned in a 1200 ml. stainless steel beaker by means of a high speed beater directly attached to a ¼ h.p. electric motor. Constructional details are adequately described and illustrated. Churning can be completed within one min. and an operator can churn and work 9-10 samples per hr. E. L. Thomas

908. A note on the electrical resistance and the keeping quality of butter. J. H. PRENTICE, Natl. Inst. for Research in Dairying, Univ. of Reading, England. J. Dairy Research, **20**, 3: 327. 1953.

Eight selected samples of butter were judged subjectively for texture defects, analyzed for

salt and moisture contents and observed for keeping quality during storage for 60 days at 70 to 80° F. Distributions of free water droplets in the butters also were estimated by means of bromophenol blue indicator papers pressed into freshly cut surfaces of the samples. In addition, two electrical properties were measured—the dielectric constant and the specific resistance. There appeared to be little relationship between keeping quality and any of the properties observed, with the exception of specific resistance. Excellent correlation between specific resistance and the onset of rancidity was observed when resistance measurements were made using a power supply which gave an output of 6 V.r.m.s. Poorer agreement was obtained when resistance measurements were made at a higher voltage (250 V.r.m.s. at a frequency of 50 cyc/sec). All low-voltage measurements were made at 200 cyc/sec, although no variation of resistance was observed with any of the samples as the frequency was varied between 150 and 1500 cyc/sec. E. L. Thomas

909. The problem of fishiness in butter. P. MUNRO and C. R. BARNICOAT, Biochem. Dept., Massey Agr. Coll., Palmerston North, New Zealand. J. Dairy Research, **20**, 3: 274. 1953.

Deliberate attempts to produce fishiness in butter under experimental conditions were unsuccessful in an investigation of reputed causative factors involving 50 churnings. Factors studied singly and in combination included low pH, high salt content, lack of pasteurization, traces of copper, and addition of borates. The butters were graded and analyzed after storage at 60-65° F. for 1-5 mo. Flavor defects such as cheesy, tallowy, metallic, and storage were common. In no case were significant amounts of either trimethylamine or trimethylamine oxide detected.

The biological and chemical theories as to the cause of fishiness are reviewed and discussed. The authors conclude that none of the proposed theories adequately account for the development of this defect in butter.

E. L. Thomas

910. A note on the vitamin D content of Indian butters. K. M. HENRY and S. K. KON, Natl. Inst. for Research in Dairying, Univ. of Reading, England. J. Dairy Research, **21**, 1: 81. 1954.

Butters were churned from herd milk collected at Bangalore and Coimbatore. Samples of the rendered fat were sent by air to Reading where they were assayed for vitamin D potency by the prophylactic method. The fats from milks collected at Bangalore during June, 1951 and April, 1952 showed a vitamin D potency of 0.56 and 0.41 i.u./g. fat, respectively. The fat from milk collected at Coimbatore during April, 1952 yielded a value of 0.29 i.u. vitaming D/g fat. These values are considerably lower than those previously reported (Indian J. Med. Res. **38**: 37. 1950) for south Indian butters and ghees. The authors report that further work is in progress in India. E. L. Thomas

911. The effect of temperature treatment of cream before churning on the consistency of butter. R. M. DOLBY, Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **21**, 1: 67. 1954.

Measurements of the hardness and "free-oil" content were made before and after storage on samples of butter from cream held at various temp. before churning.

Butter from cream held at 60° F. and churned at 45° F. was much softer and contained more free oil than butter from cream held and churned at 45° F. Precooling cream to 45° F. before being held at 60° F. resulted in butter which was initially slightly softer than that from cream held at 60° F. without precooling, but the difference disappeared after storage for 4 mo. at 14° F. Fat losses were abnormally high when cream was not precooled prior to holding at 60° F.

Slow cooling of cream to 45° F. resulted in softer butter with a higher free oil content than rapid cooling. Butter from cream held at a temp. below that at which it was churned did not differ significantly in hardness or free oil content from that produced from cream held at the churning temp. E. L. Thomas

CHEESE

912. Moisture losses in Cheddar Cheese undergoing curing. J. K. SCOTT, Dairy Research Inst., New Zealand. J. Dairy Research, **21**, 2: 212. 1954.

The loss of moisture in stored cheese is considered as a special case of drying, and the fundamentals of drying theory applied.

The rate of moisture loss (expressed as moisture loss per lb. of free moisture) is dependent on the rate of moisture movement inside the cheese, and hence a function of the group [diffusion rate × time/(height)^2]. Variables correlated by means of the above expression were time of storage, moisture content, fat content, air humidity, temp. size, and shape of the cheese.

A decrease in relative humidity from 82 to 66% resulted in a 15% increase in moisture lost from cheese during storage. At constant humidity, cheese stored at 45° F. lost about 8% less moisture than cheese at 55° F. For cheese of export size, assuming an average fat content, it is shown that the average moisture loss in lb/lb of free moisture can be taken as 1.75 (days)$^{0.52}$ at a storage temp. of 55° F. Correction factors for other temp. are given, amounting to approximately an 8% increase in moisture loss for each 10° F. rise in temp.

The fundamentals of drying theory as ap-

plied to cheese are discussed, and equations are presented showing the relation between moisture loss and the factors influencing it.
E. L. Thomas

913. Cheese slice treatment and product to prevent slice adhesion and mold. G. E. GRINDROD. U. S. Patent 2,684,906. 7 claims. July 27, 1954. Official Gaz. U. S. Pat. Office, **684**, 4: 880. 1954.

Freshly sliced pieces of cheese are subjected to a dehydration process which de-emulsifies the fat on the surface, thus preventing mold growth and adhesion of the stacked slices.
R. Whitaker

914. Methods for cheese packaging and treatment. G. GRINDROD. U. S. Patent 2,684,905. 10 claims. July 27, 1954. Official Gaz. U. S. Pat. Office, **684**, 4: 880. 1954.

The surface of cheese is heated by means of infra-red radiation to inhibit mold growth. The heat treatment causes a phase reversal of the emulsified fat to form a continuous fat surface layer. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

915. The temperature variation of the specific gravity of reconstituted skimmilk. G. BABAD, Y. LEVIN, and N. SHARON, Dairy Research Lab., Agr. Research Sta., Rehevoth, Israel. J. Milk and Food Technol., **17**, 7: 219. 1954.

The sp.g. of reconstituted skimmilk at different concentrations and temp. has been reported. A constant temp. of 15.5° C. was used, therefore the temp. variation of the sp.g. reconstituted skimmilk of 7-30% total solids in the range of 10-40° C. was made. A general formula for making the correction has been derived.

$L = (4.259 - 0.00113t - 0.000050t^2)\ TS - (1.249 + 0.0282t + 0.0046t^2)$. H. H. Weiser

916. The influence of lipids on self-dispersion and on ease of dispersion of milk powder. W. K. STONE, T. F. CONLEY, and J. M. MCINTIRE, Q.M. Food and Container Inst. for the Armed Forces, Chicago, Ill. Food Technol., **8**, 8: 367. 1954.

The rate of self-dispersion and the ease of dispersion by stirring of premium grade whole milk powders and laboratory prepared spray dried milk powders were determined under specified conditions. In water at 75° F., self-dispersion of dry whole milk was greatly increased by tempering the powder at 95 to 140° F. which caused melting of the milk fat. The temp. (50 to 120° F.) of non-fat milk powder had only a slight effect on self-dispersion in water at 75° F. With the whole milk powder tempered at 72° F., self-dispersion greatly increased when the temp. of the water was raised to the melting range of the fat or to higher temp. Non-fat milk powder, in general, showed a gradual increase in self-dispersion as the temp. of the water was raised from 35 to 150° F. As the milk fat content of milk powder increased, self-dispersion decreased. With the water at 75° F., whole milk powder tempered at 120° F. dispersed more rapidly by stirring than powder tempered at 72° F. E. R. Garrison

DAIRY BACTERIOLOGY

917. Comparison of Escherichia coli and Streptococcus faecalis as a test organism to determine the sanitary quality of food. C. H. ALLEN and F. W. FABIAN, Dept. of Bact., Mich. State Coll., East Lansing. J. Milk and Food Technol., **17**, 7: 204. 1954.

The authors suggest the usefulness of *E. coli* and *S. faecalis* as test organisms to indicate the potential danger of bacterial contamination in food. Since these bacteria are normal inhabitants of the intestinal tract and may be associated with enteric bacteria responsible for numerous food poisoning outbreaks, their presence should cause some concern, and further search for the source of contamination should be made.
H. H. Weiser

918. Comparison of Escherichia coli and Streptococcus faecalis as a test organism to determine the sanitary quality of food. C. H. ALLEN and F. W. FABIAN, Dept. of Bact., Mich. State Coll., East Lansing. J. Milk and Food Technol., **17**, 8: 237. 1954.

Viability tests were conducted on 6 strains of *E. coli* and 2 strains of *S. faecalis* when inoculated into 12 different foods ranging in pH from 2.8 to 6.7. Lauryl tryptose broth gave more positive coliform tests than the lactose broth when incubated for 16 hr.

S. faecalis survived longer in orange juice and mayonnaise at pH 3.5-3.7 than any of the strains of *E. coli*.

There was no apparent difference between the viability of the test organisms within the time limits studied in the less acid foods. In the acid foods *S. faecalis* remained viable longer than *E. coli*. H. H. Weiser

919. Comparison of boric acid and lactose broths for the isolation of Escherichia coli from citrus products. E. R. WALFORD, Fruit and Vegetable Chem. Lab., Pasadena, Cal. Appl. Microbiol., **2**, 4: 223. 1954.

A comparison of lactose broth and boric acid as a presumptive enrichment media for the isolation of *E. coli* from citrus products has been made. Boric acid broth was superior to lactose broth because of the number of positive presumptive tests, lower false positive tests and greater recovery of *E. coli*. H. H. Weiser

920. Assay of antibiotics by use of methylene blue milk. I. A. SCHIPPER and W. E. PETERSEN, Univ. of Minn., St. Paul. Am. J. Vet. Research, **15**, 56: 475. 1954.

A relatively simple method for determining concentrations of aureomycin and terramycin has been developed. It depends upon using an organism *(B. mesentericus)* that is sensitive to the antibiotics and also capable of rapid reduction of methylene blue. Suitable test organisms for other antibiotics have not yet been found. The method involves diluting the material to be assayed with a dried milk medium from 0 to 10 times, and mixing this 1:1 with methylene blue containing the test organism. An antibiotic standard is included in each series to determine the comparable dilution of material which produces the same methylene blue reduction end point. E. W. Swanson

921. Differences in the rates of deterioration of inoculated milk during summer and winter. T. J. CLAYDON, Kan. Agr. Expt. Sta., Manhattan. Appl. Microbiol., **2**, 4: 221. 1954.

Sterilized milk samples were seeded with organisms obtained from utensils, teat-cup liners, wash tank, floor water, manure, and feed on three grade-A dairy farms. The raw milk samples obtained from these farms were held at 10 and 24° C. The time required for the development of quality changes in the inoculated sterilized milk and the raw milk produced on the same farms was determined. A total of 451 samples was examined during summer and winter seasons. Quality defects were more pronounced in summer than in winter. The holding temp. of raw milk following production is responsible for the lower keeping quality during the summer. H. H. Weiser

DAIRY CHEMISTRY

922. Detection of adulteration of butter with vegetable oils by means of the tocopherol content. J. H. MAHON and R. A. CHAPMAN, Food and Drug Lab., Ottawa, Canada. Analyt. Chem., **26**, 7: 1195. 1954.

The low tocopherol level in butter oil and high level in most vegetable oils provide a basis for the determination of adulteration of butter oil with vegetable oil. A rapid procedure employing a colorimetric method for the determination of total tocopherol in butter oil is described. Synthetic butter colors and vitamin A alcohol are removed by extraction of the fat solution with 60 volume % sulfuric acid. Carotene which is not removed by this procedure must be estimated and a correction aplied. When the tocopherol value of butter oil is less than 50γ per g. it should not be considered adulterated on the basis of tocopherol value although such butter could be adulterated with lard, tallow, or coconut oil. A tocopherol value greater than 60 definitely indicates adulteration. B. H. Webb

923. Color changes in heated and unheated milk. I. The browning of milk on heating. H. BURTON, Natl. Inst. for Research in Dairying, Univ. of Reading, England. J. Dairy Research, **21**, 2: 194. 1954.

Reflectance methods were used to study the browning reaction in milk. Two different instruments were employed, a Beckman spectrophotometer with reflectance attachment, and an EEL (Messrs. Evans Electroselenium Ltd.) reflectance spectrophotometer.

Heating milk for various times at 110° C. caused an initial rise in reflectance, followed by an approximately linear fall which was exponential in nature. Reflectance changes were most pronounced at the ultra-violet and blue end of the visible spectrum. Reflectance changes were more pronounced in separated milk than in homogenized whole milk, and the reflectance of 40% cream was only slightly affected. The fat phase tends to mask the changes taking place in the other milk constituents.

Curves are presented showing the variation of reflectance of separated milk with temp. and time. The variation of the logarithm of the rate of browning with temp. is linear over the range of 95° C. to 120° C. The Q_{10} of the browning reaction was found to be 3.1 for separated milk and 2.95 for homogenized milk.

The rate of browning of heated separated milk as measured by reflectance increased with increasing alkalinity. Variation of browning rate with pH followed a pattern similar to that obtained by Patton (J. Dairy Sci., **35**: 1053. 1952) who used the trypsin-digestion method of brownness determination. Addition of small amounts of formaldehyde to milk markedly reduced the rate of browning. It was further shown that no change in color occurred in sterilized milk during storage at temperatures up to 37° C. for 11 days.

The practical application of reflectance measurements in the routine control of the sterilization process is discussed. E. L. Thomas

924. Heat-induced acidity in milk. F. H. GRIMBLEBY, Dept. of Agr. Chem., Univ. of Reading, England. J. Dairy Research, **21**, 2: 207. 1954.

Samples of separated raw milk were heated at 60, 70, 80, 90, and 100° C. for periods up to four hours and the titratable acidity, pH, formol titre and lactose were determined on the heated milks. Within the range of 60-80° C. an inverse relationship was found to exist between titratable acidity and formol titre of heated milk, and between titratable acidity and lactose content. It is suggested that the combination of lactose and protein, with the elimination of basic amino groups, is one of the main reactions responsible for the heat-induced acidity. Above 80° C., where heat denaturation and thermal decomposition of the proteins occur, heat-induced acidity developed rapidly

and was accompanied by a marked increase in the number of basic amino groups and in the number combining with lactose. Browning of the milk occurred only above 80° C. and its intensity was proportional to the temp. and time of heating. The fact that combination of lactose and protein can occur without the simultaneous production of brown color is regarded as evidence that the browning reaction proceeds in at least two stages.

E. L. Thomas

925. Effect of calcium removal by ion exchange on the properties of fluid milk. R. D. COLEMAN, S. J. BISHOV, and J. H. MITCHELL, JR., Q.M. Food and Container Inst. for the Armed Forces, Chicago, Ill. Food Technol., **8**, 5: 211. 1954.

The removal of Ca from fluid milk by ion exchange was investigated as a possible method of increasing the resistance of proteins to denaturation when milk is subjected to high temp. in order to decrease lipid oxidation in the manufacture of dry whole milk and other dairy products. Cationic and anionic synthetic exchange resins designated as Amberlites were used singly, in series and as mixtures in the demineralization of milk by the batch and column techniques. The milk was analyzed before and after treatment for ash, Ca, fat, and N and the pH determined. Flavor evaluations of the milks were made by a panel of judges.

A larger portion (up to 50%) of the Ca could be removed from milk with less change in flavor rating by using cationic and anionic resins in intimate mixture than by the use of these resins singly or in series. Change in pH was slight, even with extensive Ca removal, when the mixed-bed method was used. The effect of removing part of the Ca from milk on the resistance of the proteins to heat-induced changes was not studied. E. R. Garrison

926. A simple method for preparing crystalline rennin. N. J. BERRIDGE and C. WOODWARD, Natl. Inst. for Research in Dairying, Univ. of Reading, England. J. Dairy Research, **20**, 3: 255. 1953.

Simplified procedures for the preparation of crystalline rennin were developed as a result of greater knowledge regarding the solubility of rennin crystals along with the fact that commercial rennet of higher purity is now available. The best of four successful preparations was obtained as follows: One gal. of commercial rennet was saturated with NaCl. The supernatant liquid was decanted onto large fluted filters made from Whatman No. 3 paper and filtration was completed in 4 days. The papers containing the entrained precipitated proteins were pulped and extracted with distilled water. The extract was adjusted to pH 5.4 and again saturated with NaCl added through a rotating semi-permeable membrane. The slow addition of salt resulted in a granular protein precipitate which was centrifuged down and redissolved in 60 ml. of distilled water. It was then stored overnight in a refrigerator, during which time a good yield of crystals was obtained. Throughout the process all solutions were kept saturated with thymol. The author discusses the possible uses of crystalline rennin in research and industry. E. L. Thomas

927. Titration curves of whey constituents. M. BOULET and D. ROSE, Div. of Appl. Biology, Natl. Research Council, Ottawa, Canada. J. Dairy Research, **21**, 2: 229. 1954.

Data are presented showing the effect of varying phosphate, citrate, and whey protein concentration on the titration curves and saturation pH of artificial and natural milk sera. Titrations were made from pH 6.5 to 10.5 in the presence and absence of oxalate and the difference between the two curves was reported as "excess base."

The observed stability of Ca was found to be much greater than that predicted from the accepted solubility and dissociation constants. Using artificial sera, it was found that the precipitation of Ca is greatly impeded by citrate. In the absence of citrate, precipitation of tricalcium phosphate was complete at pH 6.0, but, in solutions containing citrate, precipitation was not complete at pH 10.0. In solutions in which considerable excess phosphate was present, precipitation of Ca ceased at about pH 9.7, even though the "excess base" consumption was less than the theoretical amount needed to precipitate all the calcium as tricalcium phosphate. Thus, it was postulated that precipitation of dicalcium phosphate must have occurred.

Significant differences were observed between titration curves for whey from fresh milk and that obtained from milk stored for three days at 40° F. E. L. Thomas

DAIRY ENGINEERING

928. Defrosting low temperature evaporators. H. H. HALLS. Ind. Refrig., **127**, 33: 34. 1954.

Energy in the form of heat must be introduced in order to rapidly defrost the evaporator. Water defrost is simply a method of introducing a liquid substance with a high thermal capacity, distributing it evenly over the entire coil, and then draining the water rapidly. Ordinary tap water meets this requirement. It is economical and can be applied to either floor or ceiling type evaporators. The nozzles for distribution of the water should be arranged on a header so the water drains away from the nozzles at the end of the defrosting cycle. For the floor type evaporator coils spray is applied at the top of the coil bank. The ceiling type evaporator utilizes a perforated pan from which the drip contacts the evaporator coils. In the pan a one inch head of water is maintained

during the defrosting operation to insure forceful drip onto the coils. A drain with a minimum pitch of one-half inch per foot is used to carry away the water. The drain should be trapped outside the refrigerated area in order to keep warm and moisture laden air from entering from this source. Where more than one unit is hooked to a common drain each individual drain must be trapped before it enters the common one. Because of the rapidity of water defrost little rise in temperature occurs in the refrigerated space because heat is localized at the coil. L. M. Dorsey

929. Apparatus and method for preserving products in sealed containers. W. McK. Martin (assignor to James Dole Engineering Co.). U. S. Patent 2,685,520. 17 claims. Aug. 3, 1954. Official Gaz. U. S. Pat. Office, **685**, 1: 175. 1954.

Liquid food products which lend themselves to continuous bulk sterilization at high temps. for short holding periods are sterilized and filled continuously into cans previously sterilized by passage through a zone of superheated steam. The filled containers are sealed with sterilized lids, the entire filling and sealing operation being conducted under aseptic conditions. A nonaqueous sterilized gas may be introduced into the equipment to provide cool filling and closing conditions.

R. Whitaker

930. Automatic pneumatic cooler door. L. L. Boyer (assignor to Knudsen Creamery Co.). U. S. Patent 2,685,376. 2 claims. Aug. 3, 1954. Official Gaz. U. S. Pat. Office, **685**, 1: 135. 1954.

An air operated automatic vertical sliding door in the wall of a refrigerated room for passage of cases of dairy products on a conveyor. An approaching case triggers the air *lift and* the departing case closes it.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

931. Plastic containers can show and protect your dairy products. Anon. Milk Prod. J., **45**, 8: 24. 1954.

Many dairy products manufacturers are using plastic containers for their products. Ice cream, cottage cheese, other soft cheeses, and dried milk products lend themselves to merchandising in plastic containers. Plastic containers are available in a wide variety of size, color, dimension, opacity, translucency, transparency, or description.

The use of plastic containers increases sales by providing that "something extra" which can often clinch a sale. Eye appeal of "see-through" packages and their reusability have tremendous sales potential. J. J. Janzen

932. New look in butter cartons. Anon. Milk Prod. J., **45**, 8: 31. 1954.

Full color pictures on packages prove effective in building more sales. The new look cartons have been instrumental in stepping up sales at regular prices. This is part of the ADA sponsored merchandising program. Fairmont Foods, Inc. officials are very enthusiastic after trying this scheme on their butter cartons. Besides full color pictures of butter's use with related items, one panel is devoted to delicious butter cookie recipes. J. J. Janzen

933. Manufacturing costs per pound of butter. Anon. Milk Prod. J., **45**, 8: 30. 1954.

Three recent bulletins dealing with the cost of manufacturing butter are discussed. The bulletins are: (1) Research Bull. 389, "The Cost of Manufacturing Butter in 13 Iowa Creameries," Iowa Agr. Expt. Sta., 1952. (2) Research Bull. 20, 1953. Idaho Expt. Sta., Moscow, (3) Station Bull. 420, Minn. Agr. Expt. Sta., University Farms, St. Paul, 1953.

These reports deal with a cross section of both small and large plants giving the price ranges accordingly. J. J. Janzen

934. Shifts in milk and cream production in Ohio. E. F. Baumer and R. H. Pollock, Ohio Agr. Expt. Sta., Wooster. Research Cir. 24. 1954.

Between 1943-1952 the number of producers selling milk to manufacturing plants decreased about 20%, or 10,000. During the same period, the number of producers selling milk to 21 Ohio fluid markets increased from 22,000 to a peak of 29,000 in 1948 and then dropped to 27,000 in 1952. Statistics for the period 1940-1950 showed that the increased demand for fluid milk resulted in a decrease of cream production and number of producers.

R. W. Hunt

FEEDS AND FEEDING

935. The magnitude of the microbial fermentation in the bovine rumen. E. G. Carrol and R. E. Hungate, Dept. of Bact., Wash. State Coll., Pullman. Appl. Microbiol., **2**, 4: 205. 1954.

The rate of volatile acid production and the total volatile acidity was measured at stated intervals in rumen contents incubated under conditions simulating those of the rumen. Acetic, butyric, and propionic acid fractions were determined. A study also was made of volatile acids produced when the animals were fed different rations. Grain-fed animals were the highest, hay-fed were intermediate and the pasturage animals showed the lowest rate of acid production. The energy available was calculated from the fermentation acids and found to be approximately 70% of the estimated total energy requirement. H. H. Weiser

936. Factors involved in forage quality for dairy calves. M. E. McCULLOUGH, Ga. Agr. Expt. Sta., Athens. Tech. Bull. 3. 1953.

A new method for determining quality of roughage is discussed. Various ratios are determined in order to obtain an evaluation number. This value is applied to the particular types of roughages studied so the user has an index to their quality. R. W. Hunt

937. The effect of tannins in Korean lespedeza and other feeds on milk production. H. A. HERMAN, G. W. GRAHAM, and K. W. BOWER, Mo. Agr. Expt. Sta., Columbia. Research Bull. 532. 1953.

Over 9,000,000 acres of Korean lespedeza are grown annually in Mo. Many farmers claim that cows on matured pastures often fall off in their production. The tannic acid in the feed is generally blamed for this "drying off" effect. However, after investigating the tannin content as affected by season, palatability, and tannin content of Korean lespedeza versus other type of roughages, it was concluded that tannins are not a factor in the decline of milk flow. More likely the lowered nutrient value and high lignin content cause a lack of available nutrients. R. W. Hunt

938. Experimental tocopherol deficiency in young calves. J. W. SAFFORD, K. F. SWINGLE, and H. MARSH, Mont. Agr. Expt. Sta., Bozeman. Am. J. Vet. Research, **15**, 56: 373. 1954.

Four calves after three colostrum feedings were fed a synthetic milk containing all known required vitamins except E, and two were fed the same diet plus 148 mg. of d-α-tocopherol acetate daily. The latter developed normally with no signs or lesions of deficiency. The unsupplemented calves showed the following symptoms beginning at the 14th day: weakness of leg muscles with a wobbly, staggering gait, getting progressively worse until the calf could not stand, and if supported it was affected by severe muscle tremors. A relaxation of the fetlocks, toe spreading, and relaxation of the shoulder muscles allowing the suprascapula to spread also developed. Some of the calves had difficulty nursing nipples because of tongue weakness. All but one had nearly normal weight gains.

Two of the deficient calves were supplemented with α-tocopherol after deficiency signs developed and showed satisfactory clinical recovery. Body temperatures, erythrocyte and leucocyte counts, and hemoglobin were not altered in the deficient calves. Heart action was affected. Electro-cardiograms showed longer P-R and Q-T intervals in the deficient calves. Gross and microscopic muscular dystrophic changes were observed in all of the deficient calves when slaughtered at 33 to 51 days. These symptoms and lesions were very similar to those which have been observed in "white muscle" disease in calves. E. W. Swanson

939. Studies of the secretion of milk of low-fat content by cows on diets low in hay and high in concentrates. II. The effects of the protein content of concentrates. C. C. BALCH, D. A. BALCH, S. BARTLETT, C. P. COX, S. J. ROWLAND, and J. TURNER, Natl. Inst. for Research in Dairying, Univ. Reading, England. J. Dairy Research, **21**, 2: 165. 1954.

Twenty cows, mainly Shorthorns, within 4 groups were randomly assigned to 5 rations, A-E, in an experiment lasting 13 wk. The control diet, A, was 18 lb. hay (lucerne, 12% crude protein) and 4 lb. (16.5% protein) concentrate per 10 lb. milk daily. In rations B-E 12 lb. hay were replaced with 6 additional lb. concentrate from the 3-9 wk. The concentrate for groups B-C was 22.3% crude protein for D-E 11.6%. In wk. 7-9 group C and D received 10 lb. delignified straw pulp (1 lb. crude fiber) daily in addition to their regular ration. All rations in wk. 1, 2, 9, and 10 were control rations; all cows were on pasture during the last 2 wks. Composite am/pm samples of milk were collected 3 times weekly for analyses.

Compared to the group on control diet the other rations did not affect total milk yield. The fat content of the milk of cows receiving low roughage decreased but the drop was less for those cows further along in lactation. Cows on the lower protein dropped further and faster than those on high protein but the differences were small. Fiber from straw apparently did not influence fat content in the 2 rations. Values for butterfat increased when roughage was returned to the ration and pasture increased the fat content and the milk yields of all groups. In the 7-9 wk. the solids-not-fat increased in the milk of cows receiving ration C and D (period of lowest fat content).

J. D. Donker

940. Studies of the secretion of milk of low-fat content by cows on diets low in hay and high in concentrates. III. The effect of variations in the amount and physical state of the hay and a comparison of the Shorthorn and Friesian breeds. C. C. BALCH, D. A. BALCH, S. BARTLETT, Z. D. HOSKING, V. W. JOHNSON, S. J. ROWLAND, and J. TURNER, Natl. Inst. for Research in Dairying, Univ. Reading, England. J. Dairy Research, **21**, 2: 172. 1954.

Sixteen Shorthorn and 4 Friesian cows were used to determine the effects of reducing the amount of hay from 16 to 4 lb. and of grinding the hay upon the fat content and yield of milk. When roughage was changed in the ration an additional amount of grain of approximately the same protein content was substituted to balance the requirements of the cows. Compared to 16 lb. of roughage, 12 lb. did not lessen the fat content of milk. The 8 and 4 lb. levels depressed the fat content. Ground hay at the 8 lb. level caused a more serious depression of fat than did the long hay. When less than 16

lb. of hay were used the decline in milk yield with time was excessive. This was checked when cows were returned to control rations. Friesian cattle behavior was similar to that of the Shorthorns. J. D. Donker

HERD MANAGEMENT

941. Effects of once-daily milking in late lactation. W. R. Hesseltine, R. D. Mochrie, H. D. Eaton, F. I. Elliott, and G. Beall, Storrs Agr. Expt. Sta., Storrs, Conn. Bull. 304. 1953.

One member from each of 5 pairs of cows was milked only once daily during the last nine weeks of a 305-day lactation to determine effects on milk production and milking time when compared with her pair-mate which continued to be milked twice daily. Cows milked once daily produced significantly less 4% F.C.M. The once-daily milked cows required about half the normal machine time. No significant differences were observed for milk from cows on once and twice-daily milking in respect to leucocytes, chloride values, or pH. R. W. Hunt

942. Farm bulk milk handling. R. P. March, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. J. Milk and Food Technol., **17**, 7: 210. 1954.

The advantages and disadvantages of farm bulk milk handling are discussed. The advantages listed include: (1) milk is measured, judged, and sampled on the farm; (2) about one lb. per cwt. or 4 to 5¢ saving in milk volume; (3) a saving of fat ranging from 0 to 5¢ per cwt.; (4) the elimination of milk cans saves about 2¢ per cwt.; (5) a reduction in hauling rates may be possible; (6) saving in labor; (7) better quality milk if properly handled; (8) highly adaptable to pipe line milkers.

Disadvantages of bulk milk handling are: (1) considerable capital is required for tank and installation; (2) freezing in some tanks may occur if not operated properly; (3) use of system may require expensive modification of milkhouse; (4) expanded hot and cold water facilities to meet greater demands for water.
H. H. Weiser

943. The cooling efficiencies and water pressures of some surface milk coolers for farms. J. K. Scott, Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **20**, 3: 280. 1953.

Three basic types of surface coolers, representative of those in use on farms were tested for cooling efficiency, allowable flow rates under various controlled conditions, and cooling water pressures for various rates of flow.

At a cooling-water ratio of 3:1, the temp. difference at the bottom of the cooler ranged from 3.3 to 7° F. for normal coolers. The allowable flow rate per sq. ft. of cooling surface varied from 33.8 to 114 lb/hr when cooling to 3° F. difference with a cooling-water ratio of 3:1. The results showed that the corrugated plate design had higher allowable capacity at low flow-rates of cooling water than the tubular designs. In all cases the cooling effect of the atmosphere was small.

At 3000 lb/hr. of cooling-water, the pressure loss was 3 lb/sq in. for the corrugated plate cooler, and appriximately 0.2 lb/sq in. per tube for the tubular coolers. The pressure loss for the tubular coolers varied approximately proportional to the square of the rate of flow.
E. L. Thomas

944. Reducing costs of raising dairy heifer replacements. L. P. Sharp and W. Sullivan, Calif. Agr. Expt. Sta., Berkeley. Cir. 435. 1954.

This bulletin supplies a plan for raising calves from birth to 24 months. Total costs (labor, feed, and calf) of raising the mature calf is approximately $255.12. R. W. Hunt

945. Livestock sprayer. C. J. Richardson and A. C. West. U. S. Patent 2,684,658. 1 claim. July 27, 1954. Official Gaz. U. S. Pat. Office, **684**, 4: 807. 1954.

A metal frame and pipe structure designed to spray cattle with insecticides as they walk through it. R. Whitaker

946. Portable milk cooler. M. M. Karr. U. S. Patent 2,685,132. 7 claims. Aug. 3, 1954. Official Gaz. U. S. Pat. Office, **685**, 1: 64. 1954.

A cooler for cooling milk on a farm is described. The cooler consists of a jacketed vertical drum which rests on a milk can and which supports a milk supply tank and strainer on top. A cover protects the milk as it is being cooled. The inlet and outlet pipes carrying the cooling liquid, are used as handles for moving the cooler from can to can. R. Whitaker

947. Milk line flushing system and valve mechanism and regulator therefor. H. A. Heckendorf (assignor to International Harvester Co.). U. S. Patent 2,685,884. 13 claims. Aug. 10, 1954. Official Gaz. U. S. Pat. Office, **685**, 2: 343. 1954.

A system of two vessels and a valve for automatically flushing the tubes of a vacuum operated milking machine. R. Whitaker

948. Self-dumping monorail live stock feed dispenser. W. W. Martin (assignor to Pacific Dairy Machinery Co.). U. S. Patent 2,685,863. 10 claims. Aug. 10, 1954. Official Gaz. U. S. Pat. Office, **685**, 2: 336. 1954.

A monorail suspended from the ceiling of the barn, provides a support for a traveling feed bin which may be automatically stopped in front of each cow and set to deliver a predetermined quantity of feed. R. Whitaker

949. Auatomatically controlled valve assembly for milking machines. A. R. HILL and J. E. JONES. U. S. Patent 2,685,862. 4 claims. Aug. 10, 1954. Official Gaz. U. S. Pat. Office, **685**, 2: 336. 1954.

A valve is described for delivering milk from a vacuum operated milker to a milk reservoir at atmospheric pressure. R. Whitaker

ICE CREAM

950. Profit through pump-and-pipe processing. J. R. FRYE, Cherry-Burrell Corp., Chicago, Ill. Food Eng., **26**, 7: 76. 1954.

At the H. A. McDonald Creamery, Detroit, batch vat-pasteurizing for ice cream making has been revamped to achieve a more mechanical operation. The core of the system is a 5000 lb. weigh-can on a dial reading scale. Fluid ingredients are pumped from remote storage tanks to the weigh-can by central control. About 10 min. are required to formulate approximately 4000 lb. of mix. Modified continuous pasteurizing is attained with a series of 3 pasteurizing vats. The homogenized and coloed mix is stored in four 1500 gal. tanks. The system has a potential output of 6000 gal. mix/8 hr. day. Substantial economies have been realized with the new layout. T. J. Claydon

951. Ice cream cabinet attachment. M. E. WEISS. U. S. Patent 2,685,980. 2 claims. Aug. 10, 1954. Official Gaz. U. S. Pat. Office, **685**, 2: 370. 1954.

A clamp with teeth for holding cylindrical bulk ice cream cans from rotating as the ice cream is dipped from the cans stored in ice cream cabinets. R. Whitaker

952. Stabilizing device for ice cream tubs. G. E. RUPPERT. U. S. Patent 2,684,172. 1 claim. July 20, 1954. Official Gaz. U. S. Pat. Office, **684**, 3: 610. 1954.

A device for holding cylindrical shaped cans of ice cream when placed in cabinets having square sleeves, consisting of a square metallic platform which fits within the sleeve and to which is attached a ring of metal with pointed tines or fins bent almost vertically upward and so spaced that the metal rim of the bottom of the ice cream carton is gripped by the tines and the can is held against rotation when the ice cream is dipped. R. Whitaker

MILK AND CREAM

953. Variations in the fat content of human milk during suckling. W. G. WHITTLESTONE and D. R. PERRIN, Ruakura Animal Research Sta., Dept. of Agr., Hamilton, New Zealand. J. Dairy Research, **20**, 2: 204. 1954.

It was found that human milk was similar to cow's milk and different from sow's milk in creaming properties. The human milk did not separate as quickly as cow's upon setting at body temperatures. There was no differential pattern in fat globule size in milk samples taken throughout the suckling period. The fat content increased on an average from 1.3% in first drawn milk to 4.1% from last milk.

J. D. Donker

MILK SECRETION

954. A recording tympanometer for the measurement of intramammary pressure in the cow. D. S. M. PHILLIPS, Ruakura Animal Research Sta., Dept. of Agr., Hamilton, New Zealand. J. Dairy Research, **21**, 2: 178. 1954.

An instrument consisting of a tympanometer to measure pressures and a continuous recording attachment is described. This device measures static as well as changing pressures in a range of 0-30 in. water. The manually operated device, placed against the wall of the udder cistern in close contact with the skin, records changes in the intramammary pressures in the lower cistern of the udder without the disturbances occasioned by direct access to the interior of the udder. J. D. Donker

955. Intramammary pressure changes in the lactating sow. II. The effects of vasopressin and acetylcholine. W. G. WHITTLESTONE, Ruakura Animal Research Sta., Dept. of Agr., Hamilton, New Zealand. J. Dairy Research, **20**, 2: 183. 1954.

Vasopressin and acetylcholine were compared to oxytocin as to the characteristic pressure curves obtained in the mammary gland of the sow. The response of the mammary tissue to vasopressin, acetylcholine, or oxytocin appears to be alike when considering the time-pressure relationship. Acetylcholine in dosages which did not cause an ejection of milk caused other responses, e.g. salivation, and in one case death from 0.2 g. Histamine (0.4 mg.), which at times caused a pronounced expansion of skin blood vessels, did not result in an ejection of milk.

J. D. Donker

956. Intramammary pressure changes in the lactating sow. III. The effects of level of dose of oxytocin and the influence of rate of injection. W. G. WHITTLESTONE, Ruakura Animal Research Sta., Dept. of Agr., Hamilton, New Zealand. J. Dairy Research, **20**, 2: 188. 1954.

Measuring the response to a given injection by intramammary pressure recordings, approximately 0.05 i.u. of oxytocin was the minimum effective amount in a 400 lb. sow. The response to this amount depended upon the injection being given in < 2 sec. There was no response if the injection period was prolonged with this small dosage. When using larger amounts (1.0 to 5.0 i.u.) there was a biphasic response with peaks of contraction about 18 sec. apart. Intermediate dosages brought about the biphasic response usually only if the injection period was prolonged. Larger amounts brought about multiple contractions independently of the injection times. J. D. Donker

NUTRITIVE VALUE OF DAIRY PRODUCTS

957. Reviews of the progress of dairy science. Section D: Nutritive value of milk and milk products. S. K. Kon and K. M. Henry, Natl. Inst. for Research in Dairying, Shinfield, England. J. Dairy Research, **21**, 2: 245. 1954.

This comprehensive review covers the three year period since the publication of the previous one (J. Dairy Res., **18**: 317. 1951). The 930 references are reviewed under the following principal headings:

I. Introduction.
II. General.
III. Nutritive value as estimated in laboratory and field experiments.
  A. Raw milk
  B. Treated milk
  C. Milk products
IV. Nutritive value of milk for man.
  A. General
  B. Milk components
  C. Effect of treatment

E. L. Thomas

958. Purified casein for nutritional research. A. M. Copping. Nature, **173**, 4416: 1165. 1954.

A brief review is given of the properties of the purified casein now being used by many laboratories engaged in nutritional research.

R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

959. Summary of known metabolic functions of nicotinic acid, riboflavin, and vitamin B_6. E. E. Snell, Univ. of Texas, Austin. Physiol. Rev., **33**, 4: 509. 1953.

Nicotinic acid occurs in biological materials in free form, as the amide, and in three coenzymatically active forms: diphosphopyridine nucleotide (Coenzyme I), triphosphopyridine nucleotide (Coenzyme II) and Coenzyme III. These coenzymes, together with appropriate apoenzymes, serve as (1) hydrogen acceptors (oxidizing agents) in the dehydration of a great variety of metabolically active compounds, and (2) phosphorylation of inorganic phosphate resulting in the formation of an energy-rich phosphate bond.

Riboflavin occurs naturally in the free form and as the coenzymes: riboflavin-5′-phosphate and flavin-adeninedinucleotide. The coenzymes may combine with apoenzymes to form flavoproteins which serve as hydrogen carriers in metabolism. They commonly link with the cytochrome system in utilizing molecular oxygen in respiration.

Pyridoxine, pyridoxamine, and pyridoxal as well as pyridoxamine phosphate and pyridoxal phosphate occur naturally. The latter is the coenzyme for a great variety of enzymes involved in catalyzing various transformations of amino acids such as degradation and synthesis. *Vitamin B_6 appears also to be in some way concerned in fat metabolism.* E. G. Moody

960. Metabolic functions of pantothenic acid. G. D. Novilli, Hardvard Med. School, Boston. Physiol. Rev., **33**, 4: 525. 1953.

Nutritional deficiency of pantothenic acid may lead to a large variety of pathological changes in most tissues of the body, affecting particularly the adrenal cortex, the nervous system, the skin and hair, and antibody production. Most, if not all, of the pantothenate in tissues is found as Coenzyme A. CoA serves metabolically by (1) its interaction with acetyl groups derived from carbohydrate, amino acids, and fatty acids from both dietary sources and the utilization of pyruvate; (2) the CoA-acetyl reactions considered as detoxication mechanisms; (3) the effect on cholesterol and steroid hormone synthesis; (4) the oxidation of fatty acids; and (5) the provision of acetyl-CoA to initiate the Krebs cycle allowing the use of a more efficient system for energy production than the glycolytic system. E. G. Moody

961. Metabolic functions of thiamine and lipoic acid. L. J. Reed, Univ. of Texas, Austin. Physiol. Rev., **33**, 4: 544. 1953.

Thiamine in the form of its coenzyme, thiamine pyrophosphate (cocarboxylase), is involved in the enzymatic decarboxylation of alpha-keto acids. Lipoic acid (6, 8-dithiooctanoic acid) is a biocatalyst for which recognized enzymatic functions and nutritional requirements have been established. However, it has not yet been demonstrated to be a dietary requirement for higher animals. The review is concerned with current research on the metabolic functions of lipoic acid and thiamine which had not previously been summarized. Topics specifically discussed were (1) the role of lipoic acid and thiamine in the oxidative decarboxylation of alpha-keto acids, (2) the role of thiamine in the generation and utilization of "active glycolaldehyde" and (3) a proposed role of lipoic acid in the light reactions of photosynthesis. E. G. Moody

962. Metabolic functions of biotin. H. A. Lardy and R. Peanasky. Univ. of Wis., Madison. Physiol. Rev., **33**, 4: 560. 1954.

The deficiency symptoms in animals—dermatitis, hair loss and muscular incoordination—do not indicate the mode of action of the vitamin. Studies with microorganisms and animal tissue preparation have brought to light a variety of carboxylation and decarboxylation reactions which are influenced by biotin. These have been reviewed. E. G. Moody

963. Antibiotics in animal nutrition. E. L. R. STOKSTAD, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y. Physiol. Rev., **34**, 1: 25. 1954.

A review is given of the literature dealing with the growth response of various antibacterial compounds such as sulfas, quaternary nitrogen compounds , and arsenicals, as well as the antibiotics. Growth responses have been reported for poultry, rodents, dogs, and swine. No effect on growth was observed for mature ruminants; calves, however, responded to the extent usually of 20-30% when receiving 15-100 mg. antibiotic daily. Penicillin was ineffective for calves. Antibiotics reportedly have a sparing action on vitamins A, B_{12}, thiamine, riboflavin, pyridoxine, choline, and certain unknown factors, Ca, NaCl, and Mn, and in mammals a protein sparing effect. It was concluded that the action of the antibiotic on growth is confined to its effect on the bacteria in the intestinal tract. One of the more plausible explanations for this effect being the inhibition of microorganisms which are deleterious because they produce toxic compounds or damage the intestinal tissues as noted by the increased response under diseased or unsanitary conditions.

E. G. Moody

964. Current concepts of the action of insulin. W. C. STADIE, Univ. of Pa., Philadelphia. Physiol. Rev., **34**, 1: 52. 1954.

The review discussed the role of insulin in (1) the transfer of glucose across the cell, (2) controlling the rate of hexose-6-phosphate formation, (3) the regeneration of ATP, and (4) the possible association with the oxidative reactions in the Kreb's cycle. E. G. Moody

965. Water and electrolyte metabolism. J. F. MANERY, Univ. of Toronto, Ont. Physiol Rev., **34**, 2: 334. 1954.

Literature pertaining to the metabolism of water and the chlorides, bicarbonates, and phosphates of Na, K, Ca, and Mg were reviewed.

E. G. Moody

966. Environmental physiology and shelter engineering. H. J. THOMPSON, D. M. WORSTELL, and S. BRODY, Mo. Agr. Expt. Sta., Columbia. Research Bull. 531. 1953.

Tabular and graphic data are presented showing effects of high and low relative humidity on weight loss, total vaporized moisture, and surface temp. of Jersey, Holstein, Brown Swiss, and Brahma cattle. Air temp. used were 12, 40, 75, 85, 95, and 100° F. At 12 and 40° F., there was no indication that increasing humidity affected vaporization rate. At 75, 85, and 95° F., there was a noticeable decrease of vaporization with increasing humidity. Individual differences and experimental variation in air temp. may have masked some of the smaller effects of humidity. Brahma cows dissipated a greater percentage of their total heat at high temp. than European cattle. Thermocouple measurements were taken on skin temp. of 15 different parts of the body. At 85° F. and above, skin and hair temp. increased somewhat on increased relative humidity, but at low temp. there was no significance. Skin temp. appeared lower in Brahmans than in European-evolved breeds at air temp. above 85° F. A striking feature of this data was that at 12° F. air temp., skin temp. of the Jersey hoof cleft was about 30° F., that of the milkwell 85° F.

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VITAMINS and AMINO ACIDS

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BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
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The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
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BACTO-NEUROSPORA CULTURE AGAR

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