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COMPARISON OF THE RATES OF IN VITRO PROTEOLYSIS IN HUMAN MILK, COW'S MILK, AND COW'S MILK PREPARATIONS BY HUMAN GASTRIC AND DUODENAL JUICES

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In reviewing the literature on the subject of comparison of the rates of proteolysis of human and cow's milk and milk preparations by usual biochemical procedures it is impressive to note that in vitro studies made with human digestive juices as the source of proteolytic enzymes are very scarce.

As studies with purified or crude enzymes obtained from other species may not be correct for determining whether or not there are significant differences in the rate of proteolysis by the human of the proteins in human and cow's milk and cow's milk preparations, further study of this problem was undertaken.

METHODS

A sample of pooled human gastric juice was obtained from six adults shortly after a light breakfast. The samples were filtered twice through cotton, placed in small containers, and kept frozen until used. The source of tryptic enzymes was a composite sample of human lyophilized duodenal juice of normal enzyme content.[1]

Nitrogen was determined by the semi-micro Kjeldahl method *(3)*. Nonprotein nitrogen was determined by mixing an aliquot of the digestion mixtures with an equal volume of 20% trichloracetic acid solution and allowing it to stand over night at room temperature. The mixture was filtered through a No. 42 Whatman filter paper, and nonprotein nitrogen was determined on the clear filtrate.

The total and nonprotein nitrogen values were determined on the milk samples and the protein content was adjusted by dilution with water to approximately the same content of protein nitrogen as human milk (170 mg/100 ml.) *(11)*. Determination of both kinds of nitrogen was necessary to maintain constant enzyme to protein ratios. Seventeen per cent of the total nitrogen of human milk and 6% of the total nitrogen of cow's milk is nonprotein nitrogen *(13)*. Eighty ml. of the diluted sample were measured into a 180-ml. electrolytic beaker, and the pH was adjusted to 2.2 by the addition of dilute HCl. To each sample were added 1 ml. toluene as a preservative and 3 ml. of glycine-HCl

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[1] Obtained through the courtesy of Dr. Paul Gyorgy.

buffer of pH 2.2 (0.1 molar). The mixture was diluted to 96 ml. with distilled water and brought to a temperature of 98° F. in a constant temperature water bath, and 4 ml. of gastric juice was added. After being mixed thoroughly, samples for analysis were withdrawn immediately and heated to boiling to stop enzyme action, and total nitrogen and nonprotein nitrogen contents and the pH value were determined. The beakers containing the digestion mixtures were covered and maintained at 98° F. for 3 hours. Samples were withdrawn at 1 and 3 hours after shaking. At the end of the 3-hour digestion, pH was again determined.

At the end of "peptic" digestion, 50 ml. of the mixtures was adjusted to pH 7.2 with 5% NaOH solution; 14.5 ml. of citric acid–disodium phosphate buffer, pH 7.2, and 4 ml. duodenal juice solution were added; the mixture was diluted to 85 ml. with distilled water and mixed thoroughly. Samples were withdrawn at once and heated to boiling, and total and nonprotein nitrogen and pH determinations made. Samples were withdrawn at ½ and 3 hours after shaking, and at the end of 3 hours the pH was determined. During both digestions the pH changes were always less than 0.3 pH unit.

The following calculations were employed:

$$TN_o - NPN_o = PN_o$$

$$\frac{NPN_t - NPN_o}{PN_o} \times 100 = \% \text{ of milk protein nitrogen liberated during time } {}_t$$

where TN = total nitrogen
PN = protein nitrogen
NPN = nonprotein nitrogen
$_t$ = time
$_o$ = zero time

Curd tension was determined by the method recommended by the Committee of the American Dairy Science Association *(1)*. The results obtained are shown in Figure 1.

RESULTS AND DISCUSSION

The design of these in vitro experiments follows the sequence of events which occurs in the gastrointestinal tract, i.e., "peptic" digestion followed by "tryptic" digestion. Human gastric juice and duodenal juice containing unpurified enzymes were chosen to reproduce as far as possible the enzyme action which occurs in vivo. The principal proteolytic enzymes of gastric juice are pepsin and cathepsin *(5, 7)*; of intestinal and pancreatic juices, trypsin, chymotrypsin, and erepsin *(4)*. The pH values of peptic digestion (pH 2.2) and tryptic digestion (pH 7.2) used in this study lie between the optimum values for the principal proteolytic enzymes of these secretions and the actual pH values of the stomach and small intestine. The pH optimum of pepsin action on casein is 1.8 *(4)*. Cathepsin is most active at pH 4.7 *(7)*. The pH of the stomach contents at the height of digestion in the breast fed infant ranges from about 2.8 to 6 with an average of 3.6 *(5, 10)*. In infants fed cow's milk the pH is higher, rang-

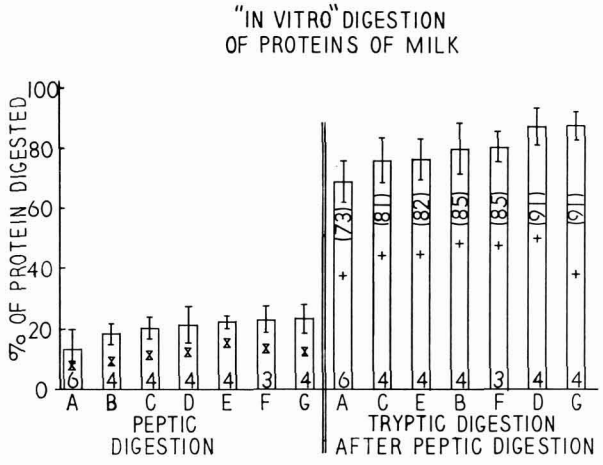

FIG. 1. Samples tested were:

A. Human milk—a pooled sample from several donors, stored at 40° C. with toluene as a preservative. Curd tension 0.

B. Commercial sterilizer-liquid infant formula, Brand A, prepared from skimmed cow's milk, lactose, and a mixture of animal and vegetable fats, protein 1.5%, lactose 7%, when diluted for feeding. Curd tension 0.

C. Commercial spray-dried infant formula, Brand A, of the same composition as B. Curd tension 3 g.

D. Commercial spray-dried infant formula, Brand B, prepared from skimmed cow's milk, lactose, and a mixture of animal and vegetable fats, protein 1.72%, lactose 6.55% when diluted for feeding. Curd tension 0.

E. Whole cow's milk sample from large pooled supply, untreated. Curd tension 25-35 g.

F. Whole cow's milk—same as E and pasteurized at 74° C. for 5 minutes. Curd tension 17-25 g.

G. Commercial sterilizer—liquid evaporated milk. Curd tension 0.

Heights of the bars indicate average per cent of the protein in milk samples digested during 3 hours. The lengths of the vertical lines at the top of each bar indicate the standard deviation. The average per cent of the protein digested during 1 hour of peptic digstion is indicated by the position of X. The average per cent of protein digested during ½ hour of tryptic digestion (after initial peptic digestion) is indicated by the position of +. Numbers at the bottom of each bar show the number of determinations. Numbers in parentheses in right hand bars indicate the per cent of total protein of the milk samples digested during both peptic and tryptic digestion periods.

ing between 4.5 and 6 *(10)*, and in adults the pH ranges from 1 to 6 with an average value of 2 for food going out of the stomach *(4)*. The optimum pH for the action of trypsin, chymotrypsin, and erepsin is around 8 *(4)*. The pH of digestion in the small intestine ranges from about 6 to 7 *(4)*.

A peptic digestion time was chosen so that under the conditions of the experiment, maximal or almost maximal pepsin action occurred. Tryptic digestion

was continued until about 85% of the protein present had been broken down. The well established method *(2)* of trichloroacetic acid precipitation of undigested protein for estimating extent of proteolysis was used.

It was of particular interest to determine whether or not the usual commercial process of heat sterilization at about 116°-118° C. for about 15 minutes causes any significant change in the rate of proteolytic digestion of cow's milk. A decrease in the biological value of milk protein subjected to heat sterilization has been observed *(6, 9)*. A decrease in biological value resulting from the heat treatment may be due in part to alteration in the rate at which various amino acids are released from the protein or formation of inter- or intramolecular bonds which are resistant to digestive enzymes *(14)*.

Heating of proteins causes both increases and decreases in susceptibility to enzymatic breakdown of milk proteins. Hankes *et al. (8)* found that the rate of release of alpha-amino nitrogen and of microbiologically available amino acids during a 2-hour digestion of casein with pepsin was unaffected by heat treatments. The rate of release of alpha-amino nitrogen during pancreatic and ereptic digestion of casein was slightly increased by autoclaving for 4 minutes and decreased by autoclaving for 20 hours. Schroeder *et al. (15)* observed a considerable increase in rate and extent of digestion of milk proteins by crystalline trypsin and chymotrypsin after autoclaving of milk at 15 lb. for 15 minutes.

Inspection of Figure 1 shows that under the experimental conditions used in this study only slight differences in rates of both peptic and tryptic proteolysis occurred among the milk samples tested. Human milk protein was found to be the least rapidly hydrolyzed of all samples tested during both peptic and tryptic digestion. The result is in accord with the finding of Mellander *(12)* that human casein is more slowly digested than bovine casein by commercial pepsin, gastric juice from infants, and commercial trypsin.

In contrast to the results of Schroeder *et al. (15)*, only slight differences in rate of tryptic digestion of raw and heat-sterilized milk were found in the present study when crude enzyme preparations from humans were used.

The differences in rates of peptic proteolysis between the spray-dried, pasteurized, sterilized, or unheated cow's milk were slight. The protein of unheated and pasteurized cow's milk digested slightly more rapidly during tryptic proteolysis than the protein of the spray-dried and sterilized samples over a 3-hour period, though the rate of tryptic proteolysis in unheated cow's milk lagged behind all other cow's milk samples at 30 minutes. The composition of the infant formulas, though different from milk, did not influence their susceptibility to proteolysis after heat-sterilization or spray-drying.

Curd tension characteristics of the samples was not a factor in the rates of proteolysis observed. Four of the seven samples had zero curd tension and one had a curd tension value of 3 g. The coagulum present in the two milk samples having significant curd tension values probably did not influence proteolysis at pH 2.2, the pH at which peptic digestion was carried out. Acidified cow's milks of pH values as high as 4.50 have zero curd tension *(10)*. Therefore, at pH 2.2, the pH of peptic digestion used here, the curd tension during digestion would

also be zero. Because the pH of peptic digestion was lower than ordinarily encountered in the stomach of infants the in vitro rates of proteolysis of the samples having curd tension values (cow's milk 25-35 g. and pasteurized cow's milk 17-25 g.) may be relatively higher than the in vivo rates which would occur in infants.

SUMMARY

In in vitro conditions under which nearly complete proteolytic digestion (as measured by decrease in nitrogen precipitable by trichloroacetic acid) occurred through the action of unpurified human digestive secretions, it was found:

1. The rates of in vitro proteolysis of human milk during both peptic and tryptic digestion (after initial peptic digestion) are slower than those of raw cow's milk or sterilized and spray-dried milk products.

2. The rates of in vitro proteolysis during both peptic and tryptic digestion (after initial peptic digestion) are essentially the same in pasteurized and unheated cow's milk as in heat-sterilized and spray-dried milk products.

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SOME PHYSICAL PROPERTIES OF MILK. I. DENSITY[1]

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Since milk is a complex biological liquid containing many physico-chemical complexes, it may be expected that some constituents will change as a result of processing. Many changes that result from processing can be determined best by chemical procedures. However, certain measurable physical changes may be more discernible and important to specific instances. Physical changes in milk or cream which may result from chemical changes in milk constituents are not clearly understood. It is hoped that more knowledge of milk constituents, particularly proteins, may be obtained through a study of physical properties. This is the first in a series of reports describing some physical changes in milk.

Two aspects of the density of milk here reported are the effects of homogenization and temperature. In 1914 Wiegner *(15)* noted that reduction in fat globule size by homogenization had no detectable influence upon specific gravity of milk. His report was later confirmed *(5)*. Many of the data, however, are somewhat limited either by the number of trials, the range of homogenization pressure, the precision of the instruments used to measure density, or the range of temperatures used in the studies.

Numerous references in the literature include some phases of the relationship of density to a change in the temperature of milk *(2, 3, 7, 8, 9)*. Most of these experiments were attempts to calculate total solids in milk based in part upon specific gravity determinations. Most of the specific gravity determinations were made with a hydrometer at temperatures below 20° C. It was believed that more precise measurements than have usually been reported might discover small differences or irregularities in densities that previously have not been detected.

The authors have been unable to find any direct evidence that milk does not have a maximum density near 4° C. According to Davies *(4)*, "the temperature of maximum density of milk is –0.3° C.," but no specific references are quoted.

PROCEDURE

The general procedure in this study was to homogenize split samples of milk at various homogenization pressures and determine densities of each split sample at eight temperatures. The procedure was repeated four times ranging from early winter to early summer.

Milk used in these experiments was mixed breed milk from the Kansas State College herd. Total solids and butterfat contents of the milks were determined,

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[1] Contributions No. 232 Dairy Husbandry Department, and No. 511 Chemistry Department, Kansas Agricultural Experiment Station.

[2] Dairy Husbandry Department.

[3] Chemistry Department.

respectively, by oven drying and ether extraction procedures with Mojonnier equipment. All of the milk studied contained 4.0 ± 0.05% butterfat and 13.0 ± 0.10% total solids.

Milks were processed with a 125-gal. per hour Manton-Gaulin homogenizer, a 300-gal. per hour Cherry-Burrell viscolizer, and a small, one-stage, laboratory homogenizer. The stainless steel laboratory homogenizer was made from a portable hand model, such as No. 7-042 in the 1954 catalog of the Fisher Scientific Co. Provision was made for a motor driven crank to lift the piston and for a coil spring to force the piston down at an adjustable but constant pressure. Pressures could be reproduced to within 1 lb. of that desired. Homogenization pressures ranging from 15 to 300 p.s.i. were obtained with the laboratory homogenizer. The milks were pasteurized at 62° C. for 30 minutes in a 200-gal. pasteurizer, cooled immediately to 59° C., and homogenized through only one stage at pressures ranging from 15 to 3,500 p.s.i. After homogenization, samples were cooled immediately in glass bottles in an ice bath and stored at approximately 2° C. until densities were measured. All samples were stored at least 18 hours except one set of samples which was held for 7 hours. These storage periods were believed to be adequate to reduce "Rechnagle effects" *(4, 10, 11, 13, 14)*.

Densities were measured with an analytical balance and a bulb made from a 15-mm. Pyrex test tube. The balance sensitivity was 0.05 mg., and weights were recorded to 0.1 mg. The bulb, which was weighted with a spiral sheet of lead, had a volume of 13.85 to 13.86 ml., depending upon the temperature. The volume of the bulb at each temperature at which densities were determined was calculated by weighing the amount of water displaced at 20° C. to 0.1 mg. A coefficient of cubical expansion of 0.00001 for Pyrex glass was used when calculating bulb volumes.

The observed weight of the bulb in air was so changed that correct densities for water would be obtained over the interval from 4° to 49° C. This change agreed satisfactorily with the correction calculated for the buoyancy of air. Lack of balance sensitivity was an instant reminder if the sample was so placed that the bulb touched the sample tube.

The bulb was lowered by a fine nichrome wire into about 32 ml. of milk contained in a 25-mm. test tube in a constant temperature bath. Times were noted when *(a)* the test tube and contents were transferred from the bath to an empty 250-ml. Erlenmeyer flask that served as a support and jacket; *(b)* the weight of the immersed bulb was determined; and *(c)* a Beckman thermometer had been transferred from the bath to the sample and a temperature reading obtained. The temperature of the milk sample and the temperature of the bath were used with the three times just listed to interpolate the temperature at the time of weighing. The time intervals were usually about 1 minute each. Temperatures were observed to 0.01° C. with Beckman differential thermometers. From Figure 1 it may be calculated that in the interval 18° to 44° C., an error of 0.1° C. would cause a density error of 0.000038 g. per milliliter.

As shown in Table 1, four samples of freshly pasteurized milk were used, representing four periods ranging from early winter until early summer. Each

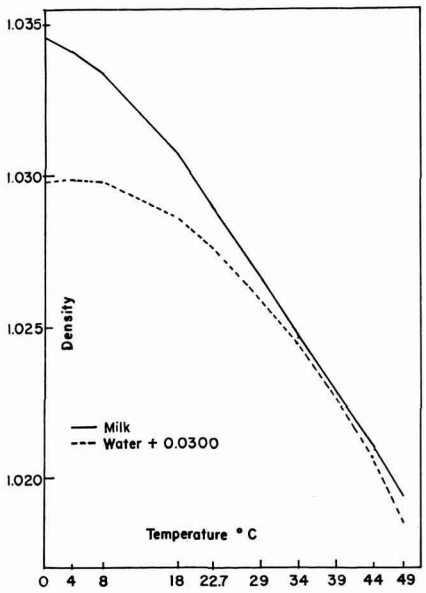

FIG. 1. The effect of temperature on the density of milk homogenized at 20 pressures ranging from 0 to 3,500 p.s.i.

sample was pasteurized and divided into at least eight fractions. Each fraction was homogenized at a different pressure. All trials included samples homogenized in the range 100-3,000 p.s.i. Density determinations were made at eight or more temperatures after cooling and holding the sample.

RESULTS AND DISCUSSION

The density measurements at each temperature of milks homogenized at all pressures were averaged for each of the four trials. The averages of the mean density readings of all milks measured at a specific temperature are marked as "average" and shown in the last column in Table 1. Mean densities of average milk used in this study ranged from 1.03463 at 0° C. to 1.02092 at 44° C. At 18° C. the mean density was 1.03071. The standard deviations $\times 10^5$ for these milks are also shown. Similar deviations for each sample at each temperature are shown after the averages for that sample. Although they are in only the fourth or fifth decimal, these deviations are large compared with the estimated precision of measurement.

Graphical illustrations of the average densities obtained at 0° C. to 49° C. are shown in Figure 1. The density of milk, unlike water, does not have a maximum at 4° C. When milks studied in these experiments were warmed from 0° C., the density of milk decreased faster than the density of water until 39° C. was reached. When milk was heated above 39° C., the density of milk decreased less rapidly than the density of water. From this observation it is evident that in

TABLE 1

The average density at various temperatures of milk receiving different homogenization treatments

Trial No.	1		2		3		4		Av.	
Date	12-9-53		1-14-54		4-14-54		6-2-54			
Fractions in each trial	11		12		20		8			
	Densities and standard deviations									
Temp. °C. when d. was measured	Density	S[a] ($\times 10^5$)	Density	S ($\times 10^5$)	Density	S ($\times 10^5$)	Density	S ($\times 10^5$)	Density	S ($\times 10^5$)
0							1.03468	17	1.03463[b]	14
4	1.03407	39	1.03397	38	1.03431	18	1.03412	17	1.03412	15
8	1.03337	35	1.03317	12	1.03362	15	1.03346	16	1.03341	15
18	1.03068	29	1.03050	12	1.03077	15	1.03087	19	1.03071	16
22.7	1.02890	16	1.02876	12	1.02907	19	1.02906	12	1.02895	15
29	1.02643	22	1.02642	18	1.02679	10	1.02674	15	1.02660	11
34	1.02460	34	1.02464	21	1.02490	7	1.02470	16	1.02471	13
39	1.02268	38	1.02275	12	1.02300	11	1.02282	31	1.02281	14
44	1.02091	34	1.02085	17	1.02012	16	1.02065	33	1.02092	26

[a] Standard deviation.

[b] Includes milks of 5-3-54 and 5-25-54.

the temperature range studied the density of milk was nearer to the density of water at 39° C. than at any other temperature.

Between 18° and 44° C. the density versus temperature curve for milk differs from a straight line by less than 0.0005 density units. Therefore, in this range the coefficient of change in density was $\frac{d_{18} - d_{44}}{44 - 18} = 0.00038$. This factor is more than twice as large as 0.00018 per degree C. which is used to adjust specific gravity readings made with a Quevenne lactometer to 15.56° C. (60° F.). However, in the range of 4° to 18° C., the temperature coefficient obtained from the milk curve in Figure 1 was in better agreement with 0.00018.

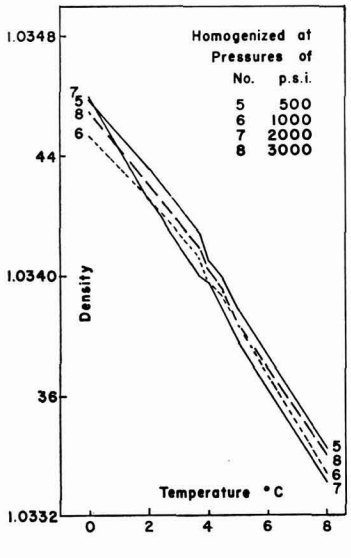

FIG. 2. The effect of homogenization upon the density of milk.

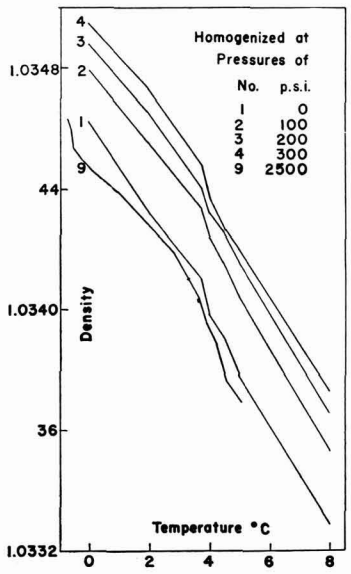

FIG. 3. Density at low temperatures of milk homogenized at low homogenization pressures.

In Figures 2 and 3 the effect of homogenization pressures on the density of milk is shown. In these figures a different scale of ordinates was required to demonstrate any effects of different homogenization processes. These density measurements were made at temperatures ranging from 0° to 8° C. Analysis of variance of the data used to compile Figure 2 indicates no simple significant relationship between density and homogenization pressures of 500 to 3,000 p.s.i. At these pressures a mean square of 50 was required for significance at the 5% level. The linear mean square value was 7 and the quadratic was 46.5. Similar analysis of variance of the data obtained when milk was homogenized at pressures of 0 to 300 p.s.i. (Figure 3) indicates that a mean square of only 36 was required for significance at the 1.0% level and 68 at the 0.1% level. The linear mean

square was 5,607 and the quadratic was 514. These analyses indicate that a highly significant increase in density occurred with increasing homogenization pressures in the range 0 to 300 p.s.i. No reason for the increase in density has been discovered. The inclusion of air in the milk samples does not appear to be a causative factor, because the densities of homogenized milks were never lower than the unhomogenized controls.

Blanchard *(1)* and Dorsey *(6)* have emphasized the anomalous behavior of water with respect to its physical properties. It was not established whether low homogenization pressure affected the extent and type of polymerization of water molecules or some other milk constituent.

It appeared that at approximately 4° C. there was an irregular change in the density of milk irrespective of homogenization pressure. This is being investigated further.

SUMMARY

During a study of some physical properties of milk, several observations were made regarding the density of milk. Unlike water, milk does not exhibit a maximum density at 4° C. However, at 4° C., there appeared to be an irregular change in density of milk.

Between 18° and 44° C., the density of milk was within 0.0005 g. per milliliter, a linear function of temperature. A temperature coefficient of 0.00038 was calculated to convert density determinations at any temperature between 18° and 44° C. to any other temperature in that range.

Homogenization of milk at pressures ranging from 500 to 3,000 p.s.i. had no significant effect upon density. However, a very highly significant increase in density of milk occurred with each increase in homogenization pressure until 300 p.s.i. was reached.

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THE RELATIONSHIP OF SERINE DEAMINATION AND HYDROGEN SULFIDE PRODUCTION BY *LACTOBACILLUS CASEI* TO CHEDDAR CHEESE FLAVOR[1]

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The ability of certain strains of *Lactobacillus casei* to deaminate serine has been reported by Kristoffersen and Nelson *(6)*. Strain 7 also produced H_2S in a V-8 juice medium without added carbohydrate. Sherwood *(7)* found that the addition of some strains of lactobacilli to milk to be used for making Cheddar cheese resulted in "sulphide" flavors. Barnicoat *(3)* reported that the presence of more than 35 p.p.m. of –SH groups per gram of cheese resulted in discoloration and off-flavored Cheddar cheese. He postulated that the presence of H_2S in New Zealand Cheddar cheese was due to lactobacilli but did not offer experimental proof.

The present paper is a report on a limited survey of the occurrence in American Cheddar cheese of *L. casei* strains capable of deaminating serine and producing H_2S. The article also contains the results pertaining to the relationship of "free" H_2S to Cheddar cheese flavor in some experimental cheese.

METHODS

The samples of cheese for isolation of lactobacilli were obtained by the method proposed by the American Public Health Association *(2)*. One g. of cheese was obtained aseptically from several places in the interior of each cheese plug and ground at 40° C. in a sterile mortar after addition of 9.5 ml. of sterile 2% sodium citrate so as to give a 10^{-1} dilution. Further dilution was carried out in the regular manner described by the A.P.H.A. The selective medium of Fabian *et al. (4)* was used for plating. Determination of H_2S production in the V-8 juice culture was made by smell and by lead acetate paper. Serine deaminase activity of the isolates was determined by the method used by Kristoffersen and Nelson *(6)*.

The nitroprusside test suggested by Barnicoat *(3)* was used for a quantitative estimation of –SH groups in the cheese. A qualitative and semiquantitative estimation of the "free" H_2S was obtained by quickly macerating some freshly cut cheese in a small meat grinder. A 25-g. sample was put into a 500-ml. Erlenmeyer flask with a 32-mm. opening, together with 100 ml. of distilled water and 4 ml. of 25% (by volume) H_2SO_4. A 4.5 × 4.5 in. piece of filter paper was held over the opening of the flask with a rubber band and moistened with a 10% solution of lead acetate. The contents of the flask were slowly brought to boiling

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and boiled for 5 minutes. The filter paper was kept moist with lead acetate solution at all times. The intensity of the black area was recorded as one to four plusses.

RESULTS AND DISCUSSION

Deamination of serine and production of H_2S by L. casei *strains isolated from Cheddar cheese.* Lactobacilli were isolated from 14 samples of Cheddar cheese made from both raw and pasteurized milk. Cheese from Illinois, Iowa, Oregon, and Wisconsin was included. The purified isolates were identified as *L. casei* strains on the basis of the following results: Gram-positive short to medium, slender rods forming short to long chains in V-8 juice broth; acid coagulation of litmus milk with reduction; optimum temperature for growth 37° C. and growth obtained at 16° C. Sugar fermentation tests were not performed.

Some of the isolates from the same cheese were obviously duplicates and were discarded. Cultures of the remainder were grown in V-8 juice medium at 32° C. for 16 hours and checked for H_2S production. The cells were then harvested and examined for deaminase activity under aerobic and anaerobic-reduced conditions at pH 5.4 and pH 8.3, with DL-serine as substrate. The results are listed in Table 1. Lactobacilli were not found in cheeses 4 and 5. The strain found in cheese 6 could not be precipitated by centrifugation, because of a layer of slime around the cells. As a reference, the results obtained with known *L. casei* strains 7, 25, 28, and 142 also are shown.

Five of the isolates from five different samples of cheese showed ability to produce H_2S in the growth medium. All five samples of cheese which contained this type of *L. casei* had a flavor score of 39.0 or better. Four of these samples were made from pasteurized milk, one from raw. None of the isolates from cheese with flavor scores below 39.0 produced H_2S. This does not necessarily mean that this type of *L. casei* was not present in the poorer cheese, as the number of colonies tested was limited, but it could indicate that the type is more numerous in good cheese.

The isolates showed varied levels of ability with regard to deamination of DL-serine. Some strains showed a deaminase activity comparable to that of *L. casei* strain 7 at both pH 5.4 and pH 8.3. Others had by the same comparison more activity in the acid range than in the alkaline. A few strains showed little activity in both ranges. The strains of *L. casei* producing H_2S did not always show the most deaminase activity. In general, anaerobic-reduced deamination conditions stimulated deamination of DL-serine by the isolates.

Even though the number of samples of cheese and isolates was small, the above results could indicate that the presence of *L. casei* strains capable of deaminating serine and producing H_2S has a relationship to the quality of Cheddar cheese. Lactobacilli could not be isolated from some of the poorest cheese, and the isolates from other poor cheese did not possess much deaminase activity. The latter was true also of some of the isolates from good cheese, although the good cheese in general also contained some strains with a rather high deaminase

TABLE 1

Production of H_2S and deamination of DL-*serine by* L. casei *types isolated from cheese and* L. casei *strains 7, 25, 28, and 142*

Cheese No. and milk type	Flavor score	Isolates	pH at harvest time	H_2S	Bacterial N per tube	NH_3 per mg. bacterial N at: pH 5.4, 52° C. Aerobic	pH 5.4, 52° C. Anaerobic-reduced[a]	pH 8.3, 46° C. Aerobic	pH 8.3, 46° C. Anaerobic-reduced[a]
					(mg.)	*(γ)*	*(γ)*	*(γ)*	*(γ)*
		1-1	4.28	0	1.76	8.7	9.3	70.3	80.8
1. Past.	39.0	1-2	4.30	0	1.55	29.7	35.5	32.4	36.6
		1-3	4.30	0	1.13	7.6	6.7	11.8	13.1
		1-5	4.27	+	1.41	7.1	7.4	36.3	49.2
2. Past.	37.5	2-1	4.25	0	1.34	0.8	3.4	6.6	3.0
3. Past.	39.5	3-1	4.68	+	1.13	17.1	26.8	53.6	148.2
		3-3	4.12	0	1.34	16.0	28.0	13.3	18.4
7. Past.	38.0	7-1	5.23	0	0.71	3.8	9.5	20.1	27.7
8. Raw	40.0	8-1	4.55	+	0.91	10.2	24.0	60.8	55.8
		8-2	4.42	0	1.34	3.2	10.7	73.6	92.5
9. Raw	37.0	9-1	4.02	0	1.27	5.8	12.2	12.6	21.4
		9-2	4.23	0	1.27	18.7	34.9	25.7	37.4
10. Raw	38.5	10-4	4.31	0	1.06	12.4	10.0	6.6	5.0
		10-5	4.45	0	1.06	2.7	26.3	95.4	146.0
11. Past.	38.5	11-7	4.80	0	0.92	−2.5	7.8	46.7	152.5
12. Past.	38.5	12-1	4.15	0	1.41	6.3	12.3	12.2	19.4
13. Past.	40.0	13-1	4.20	+	1.63	1.9	10.8	20.2	25.2
14. Past.	39.0	14-3	4.29	+	1.34	39.8	46.7	92.3	111.8
		14-4	4.31	0	1.41	−0.1	0.0	10.2	11.8
		25	4.61	0	1.20	0.0	−0.2	3.8	0.0
		28	4.38	0	1.06	6.6	8.4	16.0	24.4
Raw		142	4.32	0	1.06	5.8	10.5	16.3	78.2
		7	4.19	+	1.34	39.3	83.2	176.6	201.2

[a] Established by addition of 100 γ of L-cysteine · HCl and gassing with nitrogen.

TABLE 2

Key to the cheese within each series

Series No.	Cheese	Experimental
	a	control
I	b	control + serine
and	c	control + *L. casei*
III	d	control + *L. casei* + serine
II	a	control (*L. casei* added)
and	b	control + whey globulins
IV	c	control + potato phosphatase
V	a	control (*L. casei* added)
and	b	control + 1 × potato phosphatase
VI	c	control + 2 × potato phosphatase

activity. H_2S production could possibly be of great importance, since such strains of *L. casei* apparently were present only in the best cheese.

H_2S in Cheddar cheese and its probable relation to Cheddar cheese flavor. In view of the above findings, determinations of –SH groups and "free" H_2S were part of the analysis performed on the cheese in an experiment conducted to elucidate some of the problems concerned with flavor development in Cheddar cheese. Table 2 is a key to the experimental set-up. All the milk was pasteurized at 161.5° F. for 15 seconds. As only the results concerning –SH groups and "free" H_2S in relation to flavor are to be discussed here, a complete description of the experimental methods and results will not be attempted. The complete experiment has been discussed by Kristoffersen *(5)*.

TABLE 3

Micrograms of –SH groups and relative intensity of "free" H_2S

		1 month		3 months		6 months	
Series	Cheese	–SH per g. of cheese	Relative intensity of H_2S	–SH per g. of cheese	Relative intensity of H_2S	–SH per g. of cheese	Relative intensity of H_2S
		(γ)		(γ)		(γ)	
	a	< 5	+	5-10	++	10-15	+(+)
I	b	5	+(+)	5-10	+(+)	10+	+++
	c	5-10	++	10-15	+++	15-20	+++(+)
	d	5-10	++	10	++(+)	20	++++
	a	< 5	+	5-10	++	10-15	+
III	b	5	+(+)	5-10	++(+)	15	++
	c	5-10	++	10-15	+++	15	++(+)
	d	5-10	++	10-15	+++	15-20	++
	a	< 5	+	5-10	+	20-25	++
II	b	5	+(+)	10-15	+++	25+	++++
	c	5+	++	10-15	++	25	+++
	a	5-10	++	10-15	++	20	+(+)
IV	b	5-10	++	15-20	++++	25	++++
	c	5-10	++(+)	10-15	+++	25	+++(+)
	a	< 5	+	10-15	+++	15-20	++
V	b	5	+(+)	5-10	++	10-15	+++
	c	5	++	10-15	+++	25-30	+++
	a	< 5	+	10	++	20	++
VI	b	5	+(+)	10-15	+++	20-25	++++
	c	5	+(+)	10	++	10-15	+

TABLE 4

Flavor score and criticisms[a] of cheese at 1, 3, and 6 months

Series	Cheese	1 month	3 months		6 months	
		Flavor score and criticism	Flavor score and criticism	Intensity	Flavor score and criticism	Intensity
I	a	39.5 sl a	39.0 f, sl a	+	38.5 a, sl b	++
	b	38.5 f, sl b	38.5 a	+++	39.5	+++
	c	39.5 sl a	39.5 sl a	++	38.5 a, sl b	++
	d	39.0 sl a	38.0 a	+++	39.0 sl a	+++
III	a	38.5 f, a, sl b	39.5 a	+++	38.5 a, b, sl f	++
	b	39.0 f	39.0 sl a	++	38.0 f, b	++
	c	38.5 a	38.5 a, b	+++	39.0 a	+++
	d	38.0 a, b	38.25 a, sl b	++	38.75 a, sl b	++
II	a	39.5 f	38.5 a, sl b	++	38.0 a, m	+
	b	39.0 sl b, a	38.5 sl a, sl ferm	+	38.5 a, b	+++
	c	38.5 sl b, a	39.0 sl a	+++	39.5 sl a	+++
IV	a	38.0 a, b	38.5 b	++	38.0 b	+
	b	38.5 a, b	38.0 b, ferm	+	38.5 b, a	++++
	c	39.0 f, a	39.0 sl b, sl a	+++	38.5 a, sl b	+++
V	a	39.0 sl a	39.0 sl a, b	+++	38.5 a, b	+
	b	38.5 sl ferm	38.5 b, ferm	++	38.75 a, b	++
	c	38.25 sl a, b	38.75 sl a, b	++(+)	39.0 b	+++
VI	a	39.5 sl b	38.5 sl a, b	++	38.0 a, b	+
	b	39.0 sl a	39.0 sl a	+++	39.0 a	+++
	c	38.5 sl a, b	38.5 sl b, a	++(+)	38.5 a, b	+

[a] sl = slightly, a = acid, b = bitter, f = flat, ferm = fermented, m = musty

The results of determination of –SH groups and "free" H_2S at 1, 3, and 6 months are shown in Table 3. Table 4 contains the numerical flavor and comparative Cheddar flavor intensity scores. The intensity score, indicated as 1 to 4 plusses, was given the cheese at the 3- and 6-month examinations, because some of the cheese was found to possess comparatively more Cheddar flavor than the difference in the numerical score indicated.

The amounts of –SH groups and "free" H_2S increased as the cheese ripened. The increase in "free" H_2S is not always apparent from the data in Table 3. The results as recorded are relative within the series and examination period but should not be compared directly from period to period. The occasional decrease in the number of plusses from one period to the next for some cheese was due to a shift in the intensity compared to the other cheese in the same series.

The concentration of –SH groups and the relative concentration of "free" H_2S corresponded at the 1-month examination. As the cheese matured, and particularly at 6 months, some variation was apparent in this relationship. Some cheese with lower concentrations of –SH groups contained more "free" H_2S than did cheese in the same series with higher concentrations of –SH groups. Other cheese contained comparatively much more "free" H_2S than did cheese in the same series with a similar or slightly lower –SH group content.

By comparing Tables 3 and 4 it is seen that the cheese containing the most "free" H_2S at 6 months received the highest flavor intensity score. The control cheese usually contained less of both –SH groups and "free" H_2S at all examina-

tion periods, indicating less bacterial activity. However, not until the sixth month was the cheese with serine (except Series III) and phosphatase preparations added found superior in both flavor score and flavor intensity. This could indicate that whereas H_2S probably plays an important part as a flavor constituent of Cheddar cheese, adding to both aroma and flavor, true Cheddar flavor does not come to a maximum until the right mixture of degradation products has been attained.

SUMMARY

Lactobacillus casei types were isolated from several samples of Cheddar cheese. Types capable of producing H_2S in the growth medium were found only in cheese with a flavor score of 39.0 or better. The ability of the isolates to deaminate serine varied, but the strains showing the highest activity were isolated from the better samples of cheese.

Some experimental cheese was examined for –SH groups and "free" H_2S. Both of these values increased as the cheese matured. At 6 months the cheese containing the highest relative concentration of "free" H_2S received the highest Cheddar flavor intensity score.

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ADDENDUM

The results of the above study pointed out the desirability of having a method for quantitative determination of "free" H_2S in cheese. Such work was begun but had to be temporarily halted because of a change of position by the senior author.

Almy *(1)* developed a colorimetric method for determination of H_2S in meats and fish. When this method was applied to cheese, it was found necessary to recover the H_2S by steam distillation rather than by gassing with CO_2. Freshly cut Cheddar cheese was passed through a small meat grinder and 100 g. was added to a 1-l. distillation flask, together with 2 ml. of mineral oil, 200 ml. of distilled water, and 12.5 ml. of 25% (by volume) H_2SO_4. This mixture was steam distilled and the distillate was collected in a 100-ml. volumetric flask containing 15 ml. of 2% zinc acetate. When the volume reached 90 ml., which took about 15 minutes, the receiver was removed. A second fraction was collected and used as a blank. After distillation was completed, 5 ml. of *p*-aminodimethylaniline and 1 ml. of 0.02 *M* $FeCl_3$ were added, and the color was allowed to develop. After 2 hours the contents of the flasks were made to volume with distilled water and the intensity of the color was measured on a Klett-Summerson photoelectric colorimeter with a 600 mμ wave-length filter. The blank was subtracted from the sample and the amount of H_2S was determined from a standard curve prepared according to Almy.

TABLE 5
Recovery of H_2S by steam distillation

Sample	Treatment	Klett-Summerson readings	Parts per billion of H_2S
2 ml. H_2S solution	measured	37	14
5 ml. H_2S solution	direct	84	37
Blank		10	—
2 ml. H_2S solution	steam	44	15
Blank	distilled	14	—
5 ml. H_2S solution	steam	87	36
Blank	distilled	13	—
100 g. Cheddar cheese	steam	57	19
Blank	distilled	20	—
100 g. Cheddar cheese + 2 ml. H_2S solution	steam distilled	92	34
Blank		24	—
100 g. Cheddar cheese + 5 ml. H_2S solution	steam distilled	120	48
Blank		26	—

Table 5 contains the results of a recovery experiment. Distillation of a known solution of H_2S resulted in values that compared favorably with direct measurements on the same solution. When the known solution of H_2S was added to Cheddar cheese and distilled, approximately 100% was recovered when the lower amount was added but only about 86% with the higher amount. Probably the concentration of H_2S should be kept below 50 parts per billion when a 100-ml. volumetric flask is used. This was also evident from the standard curve, which flattened out at 250 parts per billion (500-ml. volumetric flasks used).

Other results indicated that all of the H_2S was distilled over in the first fraction. Equal values were obtained on the second through the sixth fraction. The 19 parts per billion of H_2S per 100 g. of cheese listed in Table 5 was the highest value encountered. This Cheddar cheese was about 10 months old. A sample of fresh curd after salting showed 2 parts per billion, and other samples gave values between these.

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DAIRY FARM SCORE CARD REVISION

Report of the Regulatory Advisory Committee of A.D.S.A. (1955)
A. C. DAHLBERG, J. T. MILES, AND W. M. ROBERTS, *Chairman*

One of the necessary objectives of the dairy industry is to supply consumers with safe, sanitary, nutritious milk and dairy products that have good flavor and eye appeal. The industry must be able to do this at a cost that will encourage farmers and processors to provide an adequate supply.

The regulatory agencies are concerned about the amount of milk consumed because it is so closely related to the health of the people. However, their primary objective is not one of economics but rather one that will insure that the milk supply is safe, sanitary, and nutritious.

Many organizations (educational, regulatory, medical, and industrial) have contributed greatly to these objectives of the dairy industry and the regulatory or health agencies. The American Dairy Science Assoc. through its members has been among the foremost contributors. The success of these joint efforts is manifested in the fact that an abundant supply of safe high quality milk and dairy products is available and constitutes the most important single group of foods in the American diet. However, in a constantly changing economy, an industry cannot remain static and rest on its laurels; it must be dynamic and keep abreast of the times. Certainly, there is every intent on the part of the dairy industry to rise to ever greater heights of performance.

Actions designed to improve the safety and quality of milk have dealt primarily with facility and method requirements for production (on the farm) and processing (in dairy plants) and product standards. Attempts have been made to relate the facility and method requirements as closely as possible to product quality. The facility and method requirements and product standards have been revised as research and technological information became available that would justify change. Also, it is recognized that in an industry as dynamic as dairying, there will continue to be changes.

To give some specific illustrations of the difficulties involved in writing standards or regulations, one might refer to such factors as healthy cows, types of barns, and cleaning methods. There is no question that it would be desirable to have milk from healthy cows. However, there is considerable difference of opinion as to what constitutes a satisfactory requirement pertaining to tuberculosis, brucellosis, and mastitis control. Also, a herd might be examined one day and be passed as acceptable and the next, some incidence of disease occur. This does not mean that it would necessarily be deleterious to health, but certainly it would be undesirable to have the milk included in the supply. Although it might be desirable to develop tests that would differentiate between disease producing and non-disease producing bacteria, it would be a physical and economic impossibility to test all of the milk supply if such tests were available. Therefore, the agencies concerned have tried to make a practical and reasonable approach by using the best research information available to establish requirements on facilities, methods, and tests that would assure a satisfactory supply of milk.

There is perhaps more disagreement concerning the requirements on barns than on any other item. This is to be expected because the barn is indirectly related to the quality of the milk. In dry arid climates the same type of barn is not needed as in climates with wet cold seasons. Even in the same area, the requirements may vary with summer and winter conditions. The human factor cannot be ignored since one person with certain habits and methods can produce better milk under a tree or in an open field than an undesirable person with the most elaborate barn. The health of the cow must also be considered. It is doubtful whether there are sufficient research data available from all sections of the country to indicate how much and what type of ventilation would be most healthful, how much space per cow is needed, how much natural and artificial light is necessary, what are the best and most economical building materials, what is the best arrangement, and so on ad infinitum. Future research undoubtedly will shed much light on these questions, but we do know now that we must have a clean protected place in which to milk. This means an impervious, cleanable floor; walls and ceilings that will protect from the weather, animals, and extraneous contamination; and adequate ventilation and light. Certainly, as additional information is obtained that will pinpoint rather closely some improvements that should be made in housing requirements, the standards must be changed.

Tremendous improvement in cleaning and sanitizing materials and methods has been made in recent years. However, it should be remembered that all new materials and methods should be thoroughly tested under laboratory and field conditions before being approved. Otherwise, some very serious damage to the industry might result.

Therefore, by way of summing up the manner whereby requirements are established, one might say that by using all of the knowledge and experience that is available and recognizing that there is still much to learn, those concerned have tried to develop practical and reasonable standards. The present status of the dairy industry proves the wisdom of their decisions.

Once facility, method, and product standards have been developed, there arises the need for some means of evaluating compliance by the industry. There are those who sincerely believe that testing of the product is sufficient. Others would place major emphasis on facilities and inspection of these facilities. The most widely accepted view recognizes that a combination of the beliefs expressed above is the most practical means of obtaining and maintaining a safe, sanitary, nutritious milk supply. A dairy farmer must have certain basic facilities and use approved methods if his milk is to comply consistently with the standards. Since it is not practical to supervise every milking, then the milk should be tested regularly at the plant. Conversely, since it is impossible to test completely every sample of milk delivered to the plant, some supervision of the facilities and methods on the farm will provide information that will reduce the number of samples that need to be tested. Visiting the farms also provides the opportunity for helping the producer maintain compliance with the standards.

The amount of detail needed in the requirements is dependent primarily upon the caliber of the supervisory personnel. If the supervisor is competent and thoroughly understands his work, a series of check items is usually sufficient. On the other hand, it is practically impossible to explain requirements in sufficient detail for untrained and incompetent supervisors. Generally, as requirements become more specific and detailed, they become less flexible. Therefore, it would seem more desirable for the dairy industry to support a stronger program of selection and training for regulatory personnel rather than be burdened with a large number of detailed nuisance requirements.

Since it is recognized that supervision of facilities and methods on the farms is desirable, it is accepted that some type of score card would be most helpful to the supervisors and the dairy farmers. Numerous score cards have been developed and are being used with good results. In 1908, the American Dairy Science Assoc. developed a score card for dairy farms. This score card was later adopted by the Bureau of Dairy Industry, U.S.D.A., and carried the designation "Approved by the American Dairy Science Association." A request was received from the Bureau to have the score card revised and approved and a committee from the American Dairy Science Assoc. was appointed to work with the Bureau representatives on it. The committee felt that a complete revision was necessary to bring the interpretation and method of scoring up to date. It was also thought that a score card should be designed with certain objectives in mind. The objectives and proposed score card follow.

OBJECTIVES

1. The sanitation report should be designed with certain psychological or educational featuers whereby the dairy farmer would be convinced that he is being given fair and reasonable treatment. It is believed that this objective will be greatly facilitated by incorporating such features as *(a)* changing the name from score card to sanitation report, *(b)* referring to rejecting milk rather than degrading, *(c)* approving facilities but giving no numerical score, so as to avoid comparisons between accepted and very elaborate or often impractical facilities, *(d)* permitting warning a farmer by checking items on the report without compulsory rejection on the next inspection, and *(e)* providing sufficient value to each item so that the sanitarian can make a substantial reduction in score if it becomes necessary to reject the milk.

2. The report should list the items that are considered essentials of sanitary milk production.

3. The report should be in sufficient detail to be of educational value to the average dairyman so there will be a minimum of misunderstanding between him and the sanitarian on the evaluation of facilities and methods.

4. The report and method of scoring should be simple and easily understood.

5. The report should recognize the importance of facilities but realize that methods are the area of greatest potential for improving milk.

6. Major emphasis should be placed on the facilities and methods that are most closely associated with the production of safe, sanitary, nutritious milk.

7. The report should reflect the general sanitary conditions under which the milk is produced so that if the average consumer were to visit a dairy farm that scores high, he would gain the impression that this is a good milk producer.

8. A numerical incentive should be given to the producer to improve his methods. A numerical evaluation of certain items would impress upon the producer the relative importance of each item.

9. The report should be arranged in the order in which the sanitarian usually checks the facilities and methods.

10. Some information should be available on the report to indicate the previous performance of the dairy farmer.

ACKNOWLEDGMENTS

The Committee would like to express its appreciation for the assistance furnished by many members of the American Dairy Science Association; P. R. Elliker, who was a member of the original committee; Fred M. Grant and George Holm, U. S. Department of Agriculture; John Faulkner and Harold Robinson, of the U. S. Public Health Service; and Daryl Taylor, U. S. Public Health Service, and Don Race, Dairy Products Improvement Institute, who made many suggestions and tested the score card under field conditions.

DAIRY FARM SANITATION REPORT

Farm No. _____ Owner _____

Address _____ Person Interviewed _____

Time _____ A.P.M. Date _____ Milk Grade _____

Delivered to _____ No. Gallons Daily _____

FACILITIES REQUIRED*

1. Health of Cows _____
   - Date Tuberculin tested _____
   - Date Accredited _____
   - Date Brucellosis tested, if required _____
   - Date Brucellosis approved _____
   - Date Veterinary examination, if required _____
2. Milking Area
   - Stanchion Barn** _____
   - Milking Parlor** _____
3. Milk House or Room _____
4. Utensils and Equipment _____
5. Cooling facilities _____
   - Method Used** _____
6. Water Supply _____
7. Toilet and Sewage Disposal _____

SANITARY METHODS

	Perfect Score	Rating Grade***	Rating Score
8. Milking Procedure:			
Milking inspected** _____ Method used:** Hand _____			
Machine _____ Pipeline Milker _____.			
Cows clean _____ Flanks, udders, and tails clipped _____			
Milker's hands clean _____ Mastitis test: kind _____			
used _____ Abnormal milk not sold _____ Milk moved			
immediately to milk room _____ Clean clothes _____			
Appearance and health of dairyman _____			
_____	20	___	___

* Check (×) only in cases of noncompliance.

** These items are for information only.

*** E = Excellent; G = Good; P = Poor; U = Unsatisfactory.

9. Milking Area: Walls and ceiling clean___ Floors and gutters clean___ Bedding ample and clean___ Manure removed daily___ Inaccessible to cows___ Free from flies___ Free from other animals___ Barnyard clean___ well-drained___ Loose housing properly maintained_____	15	___	___
10. Milk House or Room: Clean___ Free from flies___ Used for milk handling only_____	10	___	___
11. Utensils and Equipment: Clean___ Properly stored___ Approved procedure used for sanitizing utensils and milking machines_____	25	___	___
12. Cooling Milk: Temperature of cooling medium___° F. and/or milk ___°F. Milk delivered to plant at 50° F. or less___ or delivered to plant within 2 hr. after milking if approved___ Cooling medium sanitary_____	15	___	___
13. Toilet and Sewage Disposal: Sanitary maintenance___ Sewage and waste properly disposed_____	10	___	___
14. General Premises: Neat and clean_____	5	___	___
Total Score	100	___	___
Farm Rating			

SUPPLEMENTARY INFORMATION (Last four records)

Bacterial Counts				
Raw (Method_____)	_____	_____	_____	_____
Thermoduric	_____	_____	_____	_____
Temperatures	_____	_____	_____	_____
Sanitation Scores	_____	_____	_____	_____

Remarks:_____

_____

_____ _____

Dairyman Sanitarian

FARM CLASSIFICATION

Milk for Bottling

If a dairy farm fails to have any item under "1-7 inclusive," it shall not be admitted to the market for the major grade (usually Grade A) of bottled milk. A check on any item under this heading automatically requires reinspection. Failure to make correction requires rejection of the milk for bottling until all items are brought into compliance. The dairy farm shall not be scored on Sanitary Methods unless 1-7 inclusive are in compliance.

The farms are to be scored on each item 8 through 14 with the following grades and numerical scores:

Excellent (E) = 100%
Good (G) = 80%
Poor (P) = 60%
Unsatisfactory (U) = Zero

For example, a farm would receive 80% of 20 or 16 points on item 8 if compliance was judged good (G); 60% of 20 or 12 points for poor (P) compliance; and zero for unsatisfactory (U) compliance.

The scores shall be used to classify the farms as follows:

95-100	Excellent
80- 94	Good
60- 79	Reinspect
59 or less	Immediate rejection

A score of 79 or less on reinspection requires immediate rejection. The dairy shall be brought into compliance before being reinstated.

The sanitarian may call for a reinspection on the basis of several minor defects. Serious violation of any particular item may be considered grounds for exclusion.

Milk for Manufacture

A dairy farm producing milk for manufacture into sweet cream, ice cream, cottage and related cheese, and dry and concentrated milks may be rated on this score report with these exceptions. Item 4 shall not require hot water and a wash vat in the milk room. Item 5 shall require facilities for cooling to 60° F. or less. Item 12, cooling to 60° F. or less shall be deemed satisfactory. Since methods are just as important for milk for manufacture as milk for bottling, the same system of scoring shall be used for it that is used for milk for bottling.

Interpretation of Items on Dairy Farm Sanitation Report

FACILITIES REQUIRED

1. HEALTH OF COWS

Tuberculin test. The herd shall be free of tuberculosis as provided under the modified accredited area system approved by the Animal Disease Eradication Branch, Agr. Research Service, USDA, or as determined by annual tuberculin tests.

Brucellosis test. The herd shall be brucellosis tested and under an approved plan of eradication of the Animal Disease Eradication Branch, Agr. Research Service, USDA. In areas that do not require testing for brucellosis, the local or state regulation on this item will be acceptable. However, it is recommended that all areas adopt an approved plan of brucellosis control.

Evidence of the tuberculin and brucellosis tests shall be a certificate identifying each animal, signed by the veterinarian and filed as directed by the regulatory officer.

Mastitis program. The strip cup or other approved test shall be used at each milking on each quarter. Abnormal milk or milk from suspicious or diseased quarters shall be discarded. When cows are treated for mastitis by infusion of the udder, the milk from the treated quarter(s) shall be excluded from the supply for at least 72 hours after the last treatment. When diseased quarters are detected, the affected cow shall be milked last or in such manner that infected milk will not contaminate the milking equipment that is used on the remainder of the healthy cows.

Apparent health of the herd. The milking herd shall be observed closely for evidence of disease by the milker or owner and regulatory representative. When the evidence demonstrates that it is advisable, the sanitarian may require an examination of the herd by a licensed veterinarian. The instructions of the veterinarian must be followed concerning isolation or segregation of sick animals.

2. MILKING AREA

A conventional milking barn, or milking barn or parlor and loose housing, or combined milking systems shall be provided. It shall be of such size and arrangement as to eliminate overcrowding, promote good health of the cows, and carry on normal milking operations without impairing the quality of the milk. The milking area shall have watertight floors and gutters (constructed of concrete or equally satisfactory material), graded so as to drain properly, and shall be kept in good repair. Walls and ceilings shall be reasonably smooth, tight, dust proof, and covered with a material that is easily kept clean. Sufficient light (properly distributed) shall be provided for milking during the day or night. There shall be adequate ventilation for the health of the cows and to eliminate excessive odors and moisture. When conditions warrant, a milking barn without four walls extending from floor to roof may be approved provided precautions are taken to prevent fowl, swine, etc., from gaining entrance and provided adjacent areas are constructed and maintained so as not to have an adverse effect on the quality of the milk.

3. Milk House or Room

A milk house or room shall be provided in which the cooling, handling, and storing of milk and milk products, and the washing, bactericidal treatment, and storing of milk containers and utensils shall be done. It shall be of sufficient size to provide adequate unobstructed working space and located convenient to or in the milking barn, giving consideration to sanitation features. It shall not open directly into a room used for domestic purposes. The floor of the milk house shall be concrete or equally satisfactory material and constructed in such a manner that it will drain properly. The walls and ceilings shall be tight, smooth, and covered with a material that is easily kept clean. Sufficient light shall be provided to permit necessary operations day or night. Ventilation shall be provided to eliminate odors and excessive moisture in the milk house. All openings to the outside shall be screened. The floors, walls, ceilings, windows, doors, screens, and ventilation shall be kept in good order. All doors shall open outward only and be self-closing.

4. Utensils and Equipment

All multi-use containers, equipment, and other utensils used in the handling, storage, or transportation of milk or milk products shall be made of smooth, nonabsorbent, noncorrodible, nontoxic material, shall be so constructed as to be easily cleaned, and shall be kept in good repair. Single-service strainer pads shall be used when milk is strained. All single-service articles used shall have been manufactured, packaged, transported, and handled in a sanitary manner.

Utensil storage. All milk utensils shall be left in a treating chamber until used; or left in a bactericidal solution; or stored in the milk house or racks, in such a manner as to protect them from contamination, inverting such articles as can be inverted. Storage racks should be constructed of metal and protected against rust. Strainer pads, parchment papers, and gaskets shall be kept until used, in the original package with covers closed, or stored in a suitable container or cabinet and protected from contamination.

Washing facilities. Sufficient hot water at a temperature of at least 120° F. shall be available in the milk house to wash utensils and equipment after each milking. A two-compartment wash vat shall be provided and used only for washing, rinsing, and bactericidal treatment of milk-handling utensils and equipment.

5. Cooling Facilities

Mechanical refrigeration or cold well water is acceptable for cooling milk, provided there is adequate capacity to cool the milk to 50° F. or less within 2 hours after completion of milking and for holding it at this temperature or lower until it is delivered. Where this requirement would work an undue hardship on producers and the supervising agency is assured that all of the uncooled milk would be delivered to the milk plant or receiving station within 2 hours after completion of milking, this item shall be deemed satisfied.

6. Water Supply

Water for all dairy purposes shall be from a supply properly located, protected, and operated, and shall be easily accessible, adequate, and of a safe, sanitary quality.

7. Toilet and Sewage Disposal

Every dairy farm shall be provided with one or more sanitary toilets, conveniently located, and properly constructed so that they can be properly operated and maintained to make the waste inaccessible to flies, to avoid pollution of the surface soil and contamination of any water supply.

SANITARY METHODS

8. Milking Procedure

The cows shall be free of visible dirt at the time of milking. If necessary, flanks, udders, and tails of cows should be clipped regularly to facilitate keeping the cows clean. Loose hair, dust, and extraneous material shall be removed from udders, teats, and flanks before milking. The strip cup or other approved test shall be used at each milking on each quarter. The fore-milk in the strip cup and abnormal milk shall not be sold. Abnormal milk is milk from cows treated with antibiotics within 72 hours, milk that is abnormal in

appearance, and milk that is shown by the mastitis test to be abnormal. Also, milk shall be practically free from colostrum. The milk shall be carefully protected at all times. Straining is permitted in the barn only when the barn scores high on cleanliness, ventilation, and freedom from flies and if straining is protected against contamination.

The appearance of the dairyman should be neat, his clothes clean, and his personal habits such as to produce good quality milk. He should be apparently free from disease.

When sanitary conditions are questionable, the score on milking details must be obtained by inspection during the milking operation. It is recommended that as many inspections as possible be made during the milking operation.

9. MILKING BARN

Walls and ceilings of the milking barn shall be reasonably free from foreign materials and dust. Floors and gutters to which cows have access shall be cleaned daily, preferably during the forenoon. Manure shall be removed and disposed of in such a manner as to be inaccessible to cows and to prevent breeding of flies. The milking barn shall be reasonably free of flies. Hogs, pigeons, poultry, and other objectionable animals shall be excluded from the milking barn. The barnyard shall be properly graded and drained to prevent the accumulation of muddy areas. Waste from pigpens, barns, and milk house shall not drain into the cow yard. Manure, soiled bedding, and waste feed may not be stored or permitted to accumulate in the loose housing area in such a manner as to permit the soiling of cows' udders and flanks, and the area shall be maintained in such a manner that it provides a reasonably firm footing for the animals. Excessive accumulations of waste animal feed shall be considered a violation of this item.

10. MILK HOUSE OR ROOM

The floors, walls, windows, shelves, tables, wash vats, and other milk room equipment shall be kept clean. The milk room shall be kept free of trash and articles not used in milk room work.

Approved insecticides or other effective fly-control measures shall be used to eliminate flies from the milk house. However, care must be taken to protect the milk and milk room equipment from contamination.

11. UTENSILS AND EQUIPMENT

Containers, utensils, and equipment with which milk comes in contact during milking, straining, cooling, handling, storage, or transportation shall be thoroughly cleaned after each usage. Cleanliness may be determined by sight, touch, or smell, by wiping with tissue or filter paper, and/or by other approved methods.

For storage of utensils, see *Utensil Storage* under Item 4 above.

Sanitizing utensils and milking machines. The application of any method or substance for the destruction of pathogens, and of other organisms as far as is practicable, which is effective and which does not adversely affect the equipment, the milk or milk products, or the health of the consumers shall be considered satisfactory compliance. All milk contact surfaces must be wetted by the bactericidal solution. Bactericidal sprays may be used for large equipment. Bactericidal treatment is not effective unless the surface has been thoroughly cleaned. Chemical solutions, once used, shall not be reused for bactericidal treatment of utensils for subsequent milkings.

12. COOLING MILK

The milk must be delivered to the dairy plant at 50° F. or less unless prior approval has been granted as outlined under item 5. The cooling medium shall be kept clean and free from odor.

13. TOILET AND SEWAGE DISPOSAL

The toilet shall be operated and maintained so that the waste is inaccessible to flies and cows and does not pollute the surface so that it will contaminate any water supply. Waste from milk houses and other buildings shall be disposed of properly. There shall be no evidence of human excreta in the milking barn or areas adjacent thereto.

14. GENERAL PREMISES

The premises shall be neat and clean and maintained in such a manner that a consumer would have a favorable impression should he see or visit the dairy farm.

CHEMICAL ESTIMATION OF PROGESTERONE IN THE BLOOD OF CATTLE, SHEEP, AND GOATS[1]

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During the course of studies on the hormonal control of mammary gland growth, there arose a need for estimations of the progesterone secretion rate. This was true especially in the larger domesticated animals, in which urinary metabolites of the hormone have received little attention *(4)*. Furthermore, the significance of pregnandiol determinations is at least uncertain in species in which it is excreted *(11, 21)* and its absence in the urine of goats, cattle, and horses *(1, 17)* prevents even pregnandiol estimation. Estimation of the progesterone secretion rate by replacement therapy in pregnant cattle, after ablation of the corpus luteum, provides another technique of indirect measurement *(12, 18)*. However, by this technique placental progesterone secretion is not determined. Within recent years more direct methods have been sought. Hooker and Forbes *(9)* developed a biological method which showed relatively high concentrations of progesterone in the blood. Edgar *(6)* described a chemical method of assay of blood progesterone. Marked disagreement between the biological and chemical methods was reported.

The present report describes briefly a method which was similar to that used by Edgar *(6)* and by Zander and Simmer *(20)*. It consisted of extraction and partition in different solvents, partition chromatography on filter paper, and quantitative determination by ultraviolet absorption spectroscopy. Blood samples from several types of the larger domesticated animals were examined. Additional observations were made on the rate of disappearance of exogenous progesterone from blood.

METHODS

Extraction. The hormone was extracted from the plasma of heparinized blood in an ethanol-ether mixture by the method of Butt *et al. (3)*. After preliminary fractionation between organic solvents the extracts were dried in vacuo over $CaCl_2$. The residue dissolved in about 0.1 ml. benzene was transferred, with two further washings of 0.1-ml. portions of benzene, to the filter paper for chromatography.

Partition chromatography. The system employed (petroleum ether—80% aqueous methanol) was that described by Bush *(2)* for the paper chromatography of steroids. A chromatographic chamber in the form of a cylindrical glass jar, 6 × 18 in., was immersed in a larger glass cylinder, 12 × 18 in., which served as a water bath. By means of a heating element, thermometer, thermoregulator,[2]

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[1] Contribution from the Mo. Agr. Expt. Station, Journal Series No. 1494. Approved by the Director.

[2] Fenwal, graduated dial head, Fenwal Inc., Ashland, Mass.

and motor with stirrer, the temperature variation in the water bath was kept within 0.5° of the chromatographing temperature of 34° C.

Saturation of the atmosphere was aided by covering the wall of the cylinder with filter paper, continually soaking up the mobile phase (petroleum ether, b.p. 80°-110° C.), which covered the bottom of the jar to a depth of about 2 cm. Two "windows" (1½ × 12 in.) were cut in the filter paper opposite the hanging chromatograms. A wad of filter paper was suspended centrally with its lower end dipping into 50-100 ml. stationary phase (80% aqueous methanol) in a small glass vessel. The glass cylinder was sealed with a petroleum insoluble lubricant[3] between the wall and the lid, on which a weight was placed.

Dried filter paper sheets (Whatman No. 1, 9 × 45 cm.), previously washed chromatographically with petroleum ether, were cut into 1 × 45 cm. strips with a common base at the upper end *(16)*. The starting line was about 4 cm. from the solvent and a 2-cm. length of filter paper dipped into the solvent trough containing the mobile phase. Two chromatograms were run at one time, and a descending flow of 35 cm. required about 150 minutes. Reference substances were chromatographed with the extracts, and one strip served as a filter paper blank in the estimation of progesterone by ultraviolet spectroscopy. A preliminary ascending chromatogram, according to the technique of Bush *(2)*, was used to concentrate the extracts on the starting line. On occasion the solvent front was difficult to detect so that a small amount of Sudan IV, in 95% ethanol, was applied to the paper since the red color, which traveled faster than progesterone, insured against an overrun. After an equilibration period of at least 8 hours about 15 ml. of the mobile phase at 34° C. was added to the upper trough through a hole in the glass lid, which was immediately stoppered again.

Several color reagents were used in the detection of spots on the paper chromatograms *(10)*. The position of progesterone in the extracts on the chromatogram was determined by placing the strip containing the reference substances, after treatment with dinitrophenylhydrazine, beside the strip on which the extract had been spotted. Where the rate of flow of the mobile phase was seen to vary from that of the reference strip, it was found expedient to express chromatographic mobility of a compound as the ratio of distance traversed by the compound to that traversed simultaneously by Sudan IV. This modified expression of mobility (R_I) gave values for progesterone of 0.82-0.83, consistently. Reference substances, usually progesterone and Δ4-androstenedione, showed the extent of spreading and separation of the steroids after chromatography. For the elution of progesterone from paper chromatograms the technique employed was based on the description by Edgar *(6)* of the apparatus used by Hanes and Isherwood.

Spectrophotometric estimation. Quantitative determinations were made on the eluates (95% ethanol) by measuring U.V. absorption at 240 mμ with a Beckman model DU spectrophotometer. Readings were taken from 220 to 280 mμ against 95% ethanol and compared with those of the blank from the same level of the chromatogram.

[3] Battenfeld Corp., Kansas City, Mo.

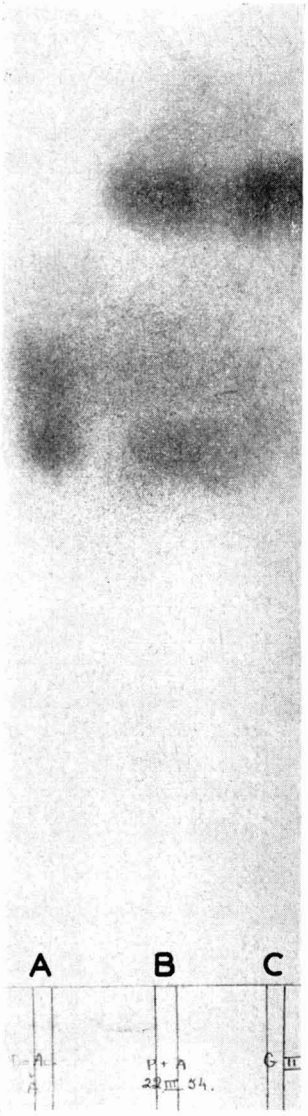

FIG. 1. Chromatogram of ovarian venous blood extract. A, desoxycorticosterone acetate and Δ4-androstenedione; B, progesterone and Δ4-androstenedione; C, progesterone from blood extract.

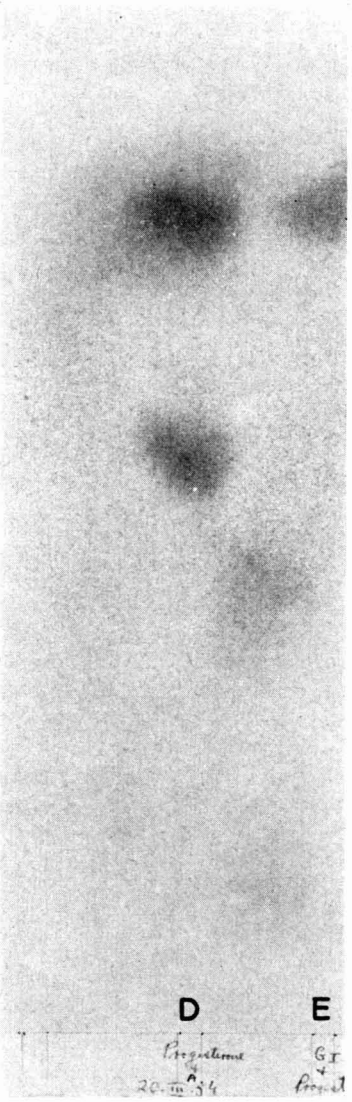

FIG. 2. Mixed chromatogram of extract from ovarian venous blood. D, progesterone and Δ4-androstenedione; E, 10 γ pure progesterone and blood extract.

Identity of the extract. Recognition of the substance as progesterone depended first on chromatographic evidence. After ultraviolet absorption spectroscopy the extract was chromatographed again and found to have the same

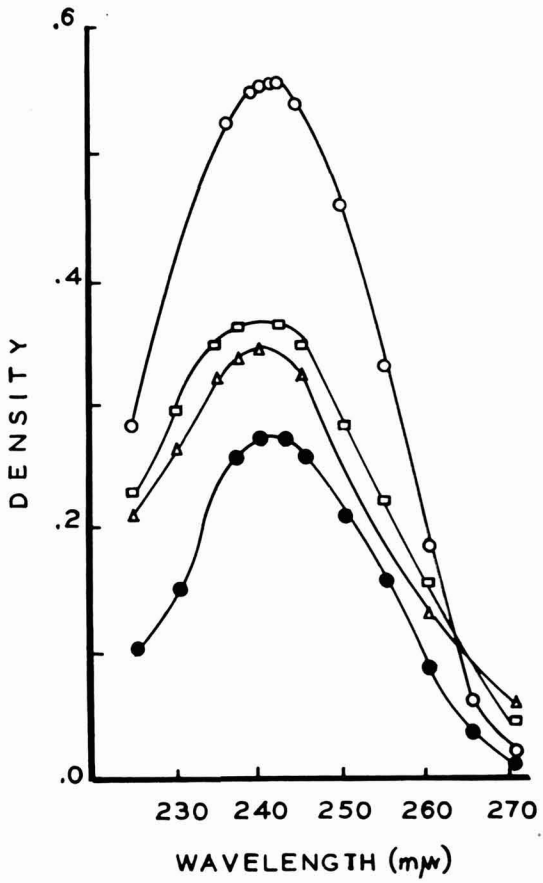

FIG. 3. Absorption spectra of progesterone and "unknown" extract from blood. ○ 10 γ prog/ml; ● 5 γ prog/ml; □ "Unknown" blood sample extract; △ Standard progesterone eluted from chromatogram.

mobility as the reference spot of progesterone (Figure 2). Color reactions for steroids on filter paper were used. Dinitrophenylhydrazine sprayed on the chromatograms gave orange-red colors, which indicated the presence of α, β-unsaturated ketosteroids. With *m*-dinitrobenzene, the 20-ketosteroids gave brown-purple colors *(16)*; this was the result with both pure progesterone and the extract being examined. As additional evidence, the absorption spectrum of the extract in ultraviolet light exhibited a close similarity to that of a solution of crystalline progesterone also in 95% ethanol (Figure 3).

RESULTS

I. *Separation of steroids by chromatography.* The separation of progesterone and $\Delta 4$-androstenedione (R_I values of 0.83 and 0.60, respectively) is shown in

Figures 1 and 2, which demonstrate the adequacy of the separation for use in the assay method.

II. *Recovery of progesterone added to plasma.* When quantities of from 10 to 40 γ of pure progesterone were added to 20-ml. samples of plasma, which were then subjected to the entire procedure, recoveries between 70 and 80% were obtained.

III. *Progesterone content of blood. (a) Peripheral blood.* No progesterone was detected in the peripheral blood from four goats, two castrated male sheep, two steers, one bull, two mature cows, and one heifer. *(b) Blood from the gravid uterine horn of the goat.* In one goat, which was unilaterally pregnant, a

TABLE 1

Plasma progesterone levels in blood from ovarian vein of a goat in late pregnancy (134th day)

Appearance of ovary	Sample size	Approximate amount	Concentration
	(ml.)	*(γ)*	*(γ/ml)*
Right ovary: 1 corpus luteum	15	35	2.3
Left ovary: 1 large follicle	15	28	1.8

sample of blood was taken from the drainage of the gravid horn. Samples from the second goat were drawn from the venous drainage of the right horn, which contained two fetuses, and from the left horn with one fetus. None of the extracts revealed the presence of progesterone, 20 ml. of plasma being used for each determination. *(c) Blood from the ovarian vein.* The examination of plasma from blood leaving the ovaries of a pregnant goat showed the presence of progesterone (Table 1). No corpus luteum was present in the left ovary, but a fairly large Graafian follicle was seen. The right ovary contained an active corpus luteum. The presence of progesterone in these extracts was substantiated by chromatographing again after U.V. absorption analysis (Figures 1, 2).

IV. *Exogenous progesterone. (a) Single subcutaneous injection.* Blood samples were taken from a 12-month-old heifer that had received single daily subcutaneous injections of 1 g. of progesterone in olive oil (50 mg/ml). It may be seen that the injection of 1 g. of progesterone produced a maximum concentration of approximately 1 γ/ml of plasma in 2 hours, which fell to one quarter of this value within 4 hours and had disappeared by 24 hours (Table 2).

(b) Continued subcutaneous injections of 1 g. of progesterone. Daily injections of 1 g. of progesterone in olive oil (50 mg/ml) were given to a pregnant

TABLE 2

Progesterone plasma levels[a] *in a heifer after single subcutaneous injections of 1 g. progesterone in olive oil (50 mg/ml)*

Experiment	Time after injection *(hr.)*					
	0	2/3	1	2	4	24
1	0	0.25	0.25	0.95	0.27	0
2					0.24	0

[a] Expressed approximately as γ prog/ml plasma.

TABLE 3

Progesterone plasma levels[a] *in a pregnant cow after daily subcutaneous injections of 1 g. progesterone in olive oil (50 mg/ml)*[b]

Day of injection	Time after injection *(hr.)*		
	½	1	24
7			0.60
			0.42
8		0.35	0.60
9	0.50		
	0.67		

[a] Expressed approximately as γ prog/ml plasma.
[b] Warmed slightly to obtain solution before injection.

cow (7 months), and blood was drawn from the external jugular vein for examination. No progesterone could be detected prior to treatment, but subsequently the hormone could be estimated at a low level in peripheral blood after 24 hours (Table 3). It appears that the daily injection of 1 mg. of progesterone maintains the level of progesterone in the blood for 24 hours at a level of approximately 0.6 γ/ml. It is presumed that this level would be exceeded at about 2 hours after each daily injection (Table 2).

A 14-month-old dairy bull was injected with 1 g. of progesterone daily for 3 days. A blood sample taken before injections were initiated showed no progesterone. Samples taken 24 hours after each injection showed the presence of progesterone; however, 48 hours after the third injection progesterone was no longer detected (Table 4). The higher levels in the bull after 24 hours may indicate that the metabolism of progesterone may be slower in males, although it had disappeared after 48 hours.

TABLE 4

Progesterone plasma levels[a] *in a young dairy bull 24 and 48 hours after daily injections of 1 g. progesterone*

Day of injection	Blood sample after injection	
	24 hr.	48 hr.
Before	none	
1	1.08	
2	1.16	
3	1.10	none

[a] Expressed approximately as γ prog/ml plasma.

(c) Continuous subcutaneous injections of 100 mg. progesterone daily. Several dairy heifers received 100 mg. of progesterone daily for variable lengths of time. At the same time 100 γ of estradiol benzoate was injected. No progesterone could be detected in the blood sample obtained 22 hours after the previous injection. Progesterone determinations were then made at short intervals after the injection. In Group I it will be noted that progesterone appeared in the blood after 30 minutes and reached a concentration of 0.5-0.6 γ/ml of plasma within 1 hour, which was maintained at the second hour but had disappeared after 4 hours (Table 5). In Group II a peak of 0.95 γ/ml of plasma was attained

TABLE 5

Progesterone plasma levels[a] *in dairy heifers having received 100 mg. progesterone subcutaneously daily*

Group No.	Time after injection *(hr.)*							
	½	1	1½	2	3	4	8	24
I	0.10	0.55		0.50		—	—	
	0.10	0.60		0.43		< 0.1		
II			0.95		0.41	< 0.1		
			0.95		0.31	< 0.1		
III				0.42		0.23		
		0.40			0.32		0.16	—
		0.48		0.48	0.25	0.25	0.15	—

[a] Expressed as γ/ml plasma.

in 1½ hours and the hormone was present only in trace amounts by the fourth hour. It appears that the injection of 100 mg. of progesterone in comparison to 1 g. may produce levels of blood progesterone in the same range although the level falls more rapidly. Thus at 4 hours little is present as compared to that present after the higher injection rate (Tables 2 and 3). In Group III the progesterone was injected as a microcrystalline suspension in water. It will be seen first that the 1-hour levels are not as high, and second that measurable amounts were present at the end of 8 hours.

DISCUSSION

Progesterone has been measured in blood from human placentas *(3, 14)* and from the ovarian veins of sheep *(6, 7)* and of the goat examined in the present work. As the corresponding peripheral blood showed no progesterone at the level of sensitivity of the chemical methods employed (approximately 0.1 γ/ml), it is possible that sufficient dilution took place that the concentrations in the circulation were below detectability. Such an explanation gains weight from the recent investigation by Salhanick *et al. (15)*, who estimated that 40 γ of progesterone was present in 618 ml. of pooled human plasma, collected between the third and seventh month of pregnancy. This finding was substantiated by Zander *(19)*, who found a progesterone concentration of 0.078 γ/ml of human blood during the second half of pregnancy, using a technique with a sensitivity of 0.05 γ/ml. Hence, it appears that progesterone may be present in very low concentrations in peripheral blood during pregnancy and yet have physiological activity.

In view of the fact that progesterone has been estimated in human placental blood, it was somewhat surprising to learn that the hormone could not be detected by the present technique in blood from the gravid horns of goats in the last month of gestation. Likewise, Edgar *(7)* recorded failure to find progesterone in blood from the middle uterine vein of a ewe 30 days pregnant. Butt *et al. (3)* have reported both positive and negative findings in the placental blood from pregnant women.

The presence of progesterone in blood from the ovarian veins of a pregnant goat, with an active corpus luteum in one ovary, was interesting in the light of the reports by Drummond-Robinson and Asdell *(5)*, and Meites *et al. (13)*. These workers concluded that corpora lutea are essential throughout all or most of pregnancy in this species. Also, progesterone was found in blood leaving an ovary containing a large Graafian follicle but no corpus luteum. These findings support the similar investigations by Edgar *(6, 7)* with pregnant ewes and point to the need for further work to establish the relative contribution of the several sources to total progesterone production during pregnancy in different species. Edgar *(6)* has discussed the disagreement between the values of progesterone concentration obtained by physicochemical methods and the higher values reported by use of the bio-assay of Hooker and Forbes *(9)*.

Three reports have dealt with a chemical determination of circulating progesterone after the administration of the hormone *(3, 8, 19)*. All recorded a high rate of elimination from the circulation when the hormone was given intravenously. Comparable results were obtained by Zarrow *et al. (22)*, who incubated blood serum containing progesterone and observed no loss of the hormone after 1 hour, in agreement with Edgar *(6)*, but almost complete inactivation within 4 hours.

From the data in Table 3 it appears that a low level of the hormone in peripheral blood was established by subcutaneous injections of progesterone for several days, in contrast to the complete disappearance within 24 hours of a single injection. One explanation of this apparent difference is that progesterone tended to crystallize out of solution at the time of the series of injections during cold weather. Hence, the animal received a suspension of minute crystals, which presumably could have resulted in a slower rate of uptake by the blood.

In a subsequent study in a young bull, plasma progesterone levels above 1 γ/ml were maintained for 24 hours for three consecutive days by daily injections of 1 g.

In heifers the subcutaneous injection of 100 mg. progesterone daily showed maximal blood levels within 1½ hours and a reduction to minimal detectable amounts within 4 hours. When microcrystals were suspended in water, the level of plasma progesterone was lower, but measurable amounts were still present at the end of 8 hours.

SUMMARY

A method is described for the determination of progesterone in blood based on extraction and partition between organic solvents, paper partition chromatography, and ultraviolet absorption spectrophotometry.

The hormone was found in a pregnant goat in blood leaving an ovary that contained a corpus luteum and also in that from one containing a Graafian follicle only. It could not be detected in the venous drainage of the uterine horns, gravid and nongravid, in two pregnant goats, nor in the peripheral blood of several of the large domesticated animals.

After a single subcutaneous injection of 1 g. progesterone the rate of disappearance from blood was examined. The data suggest a peak concentration about 2 hours after administration. Subcutaneous injection of the hormone for several days resulted in a detectable concentration in the peripheral blood of a pregnant animal. In a young dairy bull plasma progesterone levels above 1 γ/ml were maintained for 24 hours for three consecutive days by daily injections of 1 g. In dairy heifers subcutaneous injections of 100 mg. daily showed maximum levels within 1½ hours and disappearance within 4 hours.

When microcrystals were suspended in water, the maximal level attained for plasma progesterone was lower, but measurable amounts were present after 8 hours.

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SOME RELATIONSHIPS AMONG THE MAJOR CHEMICAL COMPONENTS OF THE BOVINE BODY AND THEIR APPLICATION TO NUTRITIONAL INVESTIGATIONS

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The chemical composition of the whole empty body of an animal represents a final state resulting from the influence of heredity and environment. Since, in the usual nutritional or physiological experiment, attempts generally are made to control genetic influences by the use of large numbers of animals, random allotment, replication and other devices, changes in the composition of the body (reflecting storage or loss of chemical components) become valuable criteria of response to the environmental treatments being imposed. It long has been recognized that changes in the concentration of fat, protein, and carbohydrates in the body reflect energy storage or loss and that the exchange of energy may be employed as a criterion of the energy value of feeds for maintenance, growth, and/or fattening.

Several generalizations of biological significance have arisen from studies of the composition of animal bodies. The very early and extensive experiments conducted by von Bezold *(33)* with mammals, birds, amphibians, fish, and other animals at various stages of development show that animals are composed in a manner characteristic of their species and age. Morphologically similar animals were reported to have a similar chemical composition. Moulton *(20)* pointed out that, at birth, cattle and guinea pigs are more highly developed chemically than are man, swine, dogs, and cats. He drew attention to the fact that cattle and guinea pigs are ambulant at birth and well developed physically, whereas man, swine, dogs, and cats are not. At birth, the animals of the respective groups were of similar chemical composition *(20)*. The results of these and other early investigations *(11, 13, 16, 17, 29)* led to the conclusion that the amount of water decreases and the ash and protein contents increase in the body as animals approach maturity. These changes are especially marked when fattening does not intervene.

Attention was attracted early to the reduction in water content accompanying the increased fattening of farm animals *(13, 26, 34)*. Since the fat and water contents are extremely variable (depending to a considerable extent upon the amount and kind of feed consumed) but together generally constitute 75 to 79% of the whole empty body of mammals, it appears that the fattening process is largely one of replacement of water by fat.

From a study of the chemical composition of the bodies of three steers, five sheep, and two pigs published by Lawes and Gilbert *(16)*, Murray *(23)* in 1919

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pointed out that the chemical composition becomes obvious when the fat content is known, because the nonfat matter is of the same composition regardless of the degree of fatness. Later, Murray *(24)* studied the analyses made of 56 cattle by Haecker *(11)* and of 36 pigs by Swanson *(29)*. The results of this study supported those of the earlier one *(23)* and, in addition, showed that the water content of the fat-free empty body decreases as animals age. The ratio of the percentage of protein to that of ash was practically constant for the three species studied *(24)*. At about the same time (1922), Moulton *et al. (21)* studied the fat-free composition of the bovine ranging in development from early embryonic stages to maturity and that of the human embryos on which Fehling *(9)* reported. The water content of the fat-free body of these mammals decreased rapidly from the time of conception to that of birth and thereafter decreased less rapidly until a relatively constant concentration of water was reached. In the bovine, this occurred between 5 and 10 months of age. In a later study of his and others' data on the composition of the bodies of mammals, Moulton *(20)* introduced the concept of "chemical maturity," which he defined as the age at which the concentration of water, protein, and mineral matter in the fat-free cell becomes practically constant. In a further report *(3)*, treatment was given to these data in addition to the analyses available at that time on the bodies of chickens and geese. The ages at which chemical maturity was attained by the various mammals studied were: rat, 50 days; guinea pig, 50 days; cat, 100 days; dog, 200 days; swine, 150 to 300 days; cattle, 150 to 300 days; and man, 500 to 1,000 days. Although mammals become chemically mature at different ages, the proportion of the total life span expended prior to the attainment of chemical maturity was about the same (3.9 to 4.6%) for all species investigated. Moulton *(20)* concluded, as did Murray *(24)*, that the relative fatness of animals of the same species does not influence the composition of the fat-free body. Therefore, the major effect of fattening upon the concentration of water, protein, and mineral matter in the whole empty body is that of dilution.

Recently, mice, rats, guinea pigs, rabbits, cats, and pigs were analyzed at various stages of life for certain mineral elements as well as for the proximate constituents *(28)*. Although the results obtained support to a large extent the generalizations reported by Moulton *(20)*, Spray and Widdowson *(28)* found considerable disparity among the ages at which the concentration of the individual mineral elements in the fat-free body approached constancy.

To date, there has accumulated a great amount of evidence supporting the generalization that the percentages of water, protein, and mineral matter approach constancy in the fat-free body of animals after "chemical" maturity is attained. However, the proportions of these substances are not strictly constant; the proportion of water decreases and the proportions of protein and ash increase as animals become older. Nevertheless, this generalization has led to the assumption that the water content of the "lean body" mass is a constant (generally 73.2%) and the usage of this, or a similar, value in the derivation of the concentration of fat, particularly in studies with man. This is necessitated by the lack of adequate numbers of actual chemical data on the body of man to deter-

mine the relationship of age to water content. If the degree of variation in the water content of the fat-free body found to be associated with age in other animals is an indication of that existing in man, the use of a constant factor could contribute to significant errors in certain kinds of experiments. Also, the use of a constant value would be particularly discrepant if applied to animals prior to their attainment of chemical maturity.

No attempt has been made here to review all of the data dealing with the composition of animal bodies but, rather, an attempt has been made to cite those reports which appear to have instrumented generalizations applying to livestock and particularly to cattle. In addition, the general concepts reviewed offer promise of the refinement of nutritional experiments especially those dealing with the evaluation of feed energy.

In many feeding experiments with livestock, body weight changes are employed as a criterion of response. This criterion would appear to be more susceptible to error when applied in experiments with ruminants than in those involving nonruminants. Changes in ruminal fill reflected in the body weight could result in appreciable errors, particularly in experiments of short duration. In addition, the use of body weight changes assumes that, irrespective of treatment, the weight gained or lost is of the same chemical composition. Mitchell *(19)*, in evaluating the use of body weight changes as a criterion of the nutritive value of proteins, concluded that the weight gains of growing animals vary in their content of water, protein, and fat. Therefore, the same increments in weight resulting from the consumption of different rations do not necessarily reflect equivalent nutritive effects. A study of the data reported by Watson *et al. (35)* on a comparison of urea and casein as a source of dietary nitrogen for cattle reveals that, although the animals fed urea gained only 70% as much weight as those fed casein, they gained 81% as much energy. These data demonstrate the inadequacy of body weight changes as an index of energy exchange.

At the present time, the total digestible nutrient (TDN) content (or some similar index) is the most commonly used criterion of the energy value of feedstuffs in the United States, and, as a consequence, rations are formulated on this basis. However, it is recognized that the TDN of one feed does not necessarily produce a response of the same magnitude as that effected by the same amount of TDN from another feed. The TDN intake-production response relationship is particularly discrepant in comparisons made between concentrate and roughage feeds. In addition, roughage feeds vary greatly in the response which they evoke in animals. Therefore, the problem of evaluating feeds for ruminants is considerably more complex than that of feeds (generally concentrates) for animals having simpler digestive systems. An evaluation of feeds on the basis of net energy reduces the energy-giving qualities to a common unit of measure, *e.g.*, a unit of net energy in one feed produces the same amount of a given response in an animal as that produced by a unit of energy from an entirely different feed.

The net energy values of diets and feedstuffs for maintenance, growth, and fattening may be determined by measuring the heats of combustion of repre-

sentative samples of the whole empty bodies of animals at the beginning and of those of different animals at the end of a feeding period. Protein and fat contribute almost all of the energy value of the body. [Carbohydrates generally constitute less than 0.5% of the bovine body *(2, 3, 30)* and, therefore, can be disregarded in considerations of the energy value of the body in experiments of long duration.] Since the caloric values of protein and fat in the bovine body are well established *(1, 2, 4, 5, 10)*, the energy value may be derived alternatively from data on the protein and fat contents of the whole empty body.

Several recent developments give a new significance to the data published on the composition of bovine bodies and to that of the use of body composition data as implements in experiments dealing with nutrition, physiology, and/or meat production. The chemical analyses made of 132 whole empty bodies of cattle ranging from 135-day-old fetuses to 12-year-old cows by Ellenberger *et al. (7)* greatly augment previously existing data for the purpose of studying generalizations for this species. In 1951, Kraybill *et al. (15)* reported that the water content of beef cattle bodies derived from the use of antipyrine as an indicator in a dilution technique compared favorably with that estimated from the specific gravity of the carcasses. More recently it was found that the water content of the bodies of dairy cattle estimated from the use of the antipyrine method agreed well with that determined by the slaughter-analysis technique *(36)*. The use of antipyrine for the estimation of body water in man was developed by Soberman *et al. (6, 27)*. Other methods for the indirect measurement of body water *(25)* might be applied in the bovine, though they have not yet been tested with this species. These recent findings in combination with the apparent significance of the reported generalizations dealing with body composition prompted the present study. The objectives of this investigation were: *(a)* to examine the relationships existing among the water, protein, fat, and ash contents of the bovine body, *(b)* to study the influence of age, sex, and body type upon the relationships among body components, and *(c)* to attempt to quantitate these relationships for application to nutritional experiments.

PROCEDURE

In these studies an attempt was made to utilize all data reported on the composition of the bovine body which satisfied certain specifications having nutritional significance. Only those data were selected which represented the percentages of water, fat, protein, and ash, or sufficient information from which these values could be derived, on the whole empty body basis.

The data employed were reported by Lawes and Gilbert *(16, 17)*, Jordan *(14)*, Moulton *et al. (21, 22)*, Haecker *(11)*, Trowbridge *et al. (31, 32)*, Haigh *et al. (12)*, Meigs *et al. (18)*, and Ellenberger *et al. (7)*. In addition, unpublished data provided by Emslie *(8)* were used. The analyses represented 256 cattle, of which 139 and 117, respectively, were of beef and dairy breeding. Unfortunately (from the standpoint of measuring sex and type differences in the compositional relationships), only six of the beef cattle were females and only two of the dairy cattle were males. The sex of seven dairy cattle was not reported. As a conse-

TABLE 1

Kinds, ages, and composition of cattle studied

Cattle	No.	Age *(days)*		Composition of whole empty bodies *(%)*							
				Water		Fat		Protein		Ash	
		(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)
All	256	661	(1-4,860)	62.7	(39.8-77.6)	14.2	(1.8-44.6)	18.5	(12.4-20.6)	4.5	(3.0-6.1)
Beef	139	503	(1-1,710)	61.1	(39.8-77.6)	16.0	(2.5-44.6)	18.3	(12.4-20.6)	4.5	(3.0-5.9)
Dairy	117	857	(1-4,860)	64.6	(49.4-76.8)	12.0	(1.8-29.2)	18.7	(14.8-20.4)	4.6	(3.7-6.1)
Males	135	518	(1-1,710)	60.8	(39.8-77.6)	16.3	(1.8-44.6)	18.3	(12.4-20.6)	4.5	(3.0-5.9)
Females	114	880	(1-4,860)	64.3	(49.4-76.8)	12.3	(3.3-29.2)	18.6	(14.8-20.4)	4.7	(3.7-6.1)

TABLE 2

Influence of age upon concentration of fat-free body components

Component	Concentration *(%)* (Mean ± std. dev.)	Correlation with age	Regression equations[a] and std. deviations from regression			
			Linear	Sy · x	Curvilinear	Sy · x
Water	72.91 ± 2.01	−0.46**	$\hat{Y} = 73.74 - 0.00116X$	1.786	$\hat{Y} = 77.16 + 0.00007X - 1.7585 \log X$	1.337
Protein	21.64 ± 1.53	0.44**	$\hat{Y} = 21.01 + 0.00095X$	1.380	$\hat{Y} = 18.35 - 0.00020X + 1.4101 \log X$	1.017
Ash	5.34 ± 0.95	0.43**	$\hat{Y} = 4.95 + 0.00058X$	0.864	$\hat{Y} = 4.37 + 0.00033X + 0.3079 \log X$	0.841

[a] $\hat{Y}$ = Percentage of water, protein, or ash; X = age (days).

** Highly significant.

quence, the major comparisons made involved essentially male beef cattle and female dairy cattle. Table 1 provides a summary of the number of animals of both sexes and body types and the ranges in age and the composition of the whole empty bodies studied. Correlation and regression analyses were employed in the examination of these data.

RESULTS AND DISCUSSION

The data published on the composition of the whole empty bodies of cattle represent a wide range of values (Table 1). As demonstrated in earlier studies, the percentages of fat and, to a lesser extent, those of water vary more than the percentages of protein and ash.

Reduction of the water, protein, and ash contents to the fat-free empty body basis tends to make the proportions of these components relatively constant (Table 2). For example, the coefficient of variation (standard deviation of the mean expressed as a percentage of the mean) in water content is reduced from 12.4% for the whole body to 2.7% for the fat-free body. Nevertheless, as the standard deviations from the means shown in Table 2 suggest, an appreciable variation in composition still persists. Since these values include those for all animals (ranging in age from one to 4,860 days), the standard deviations are somewhat greater than they would be for the body components of only those animals having attained "chemical maturity."

Age is highly significantly correlated with the percentages of the fat-free body components. The curvilinear equations computed were found to express the relationships between age and fat-free body constituents somewhat more accurately than the corresponding linear equations. A comparison of the standard deviations from regression with the respective standard deviations of the mean water, protein, and ash contents demonstrates that a consideration for the age of animal represents a refinement over the use of the mean fat-free components (Table 2). The relationships of age to the concentrations of water, protein, and ash in the fat-free body are shown in Figure 1. These data suggest that, in terms of Moulton's definition *(20)* of chemical maturity, the bovine is "immature" from birth until about 200 days of age, at which time it enters a transitional stage lasting until about 500 days of age, after which the animal is approximately chemically mature. As Figure 1 demonstrates, actual constancy of composition or true chemical maturity as conceived by Moulton is not reached during the first 4,000 days of life by the bovine. If chemical maturity is defined as the age at which the composition of the fat-free body becomes predictable or conforms closely to a mathematical generalization, then the bovine body is chemically mature at birth.

These observations emphasize the fallacy of the assumption that the water content of the "lean body" is a constant (such as 73.2%) even for animals relatively mature chemically.

In view of the great amount of variation in the percentages of the major components of the whole empty body and the smaller, though still considerable, variation in those of the constituents of the fat-free empty body, an attempt was

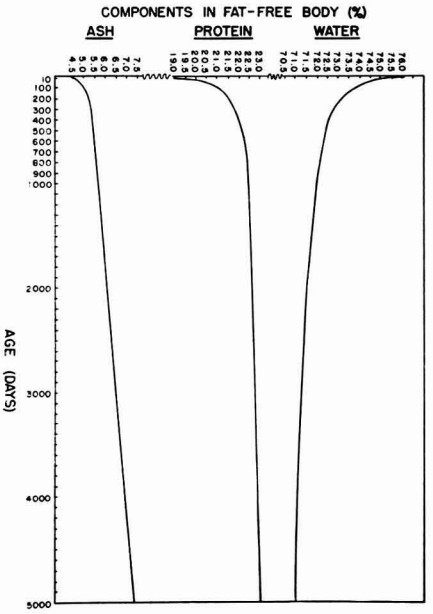

FIG. 1. Influence of age upon the water, protein, and ash contents of the fat-free bovine body.

made to determine whether or not the body components could be reduced to a group occurring in variable amounts and another group existing at a constant level. A study of the data revealed that, of the percentages of the major components, those of fat and water in the whole empty body are variable and those of protein and ash in the fat-free, dry substance are practically constant. Since

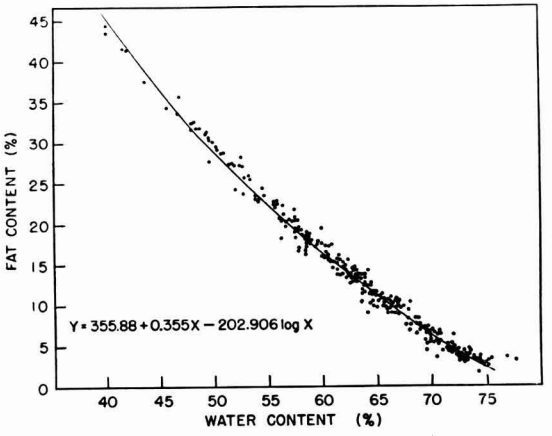

FIG. 2. Relationship between the concentrations of fat and water in the whole empty body of the bovine.

TABLE 3

Relationships between the water and fat contents of the whole empty bodies of cattle

Cattle	Correlation coefficients[b] (± Std. error)	Regression equations[c] and std. deviations from regression			
		Linear	Sy · x	Curvilinear	Sy · x
All (256)[a]	−0.987 ± 0.0017	$\hat{Y} = 84.29 - 1.1182X$	1.440	$\hat{Y} = 355.88 + 0.3550X - 202.91 \log X$	1.029
Dairy (117)	−0.986 ± 0.0026	$\hat{Y} = 78.11 - 1.0228X$	1.143	$\hat{Y} = 388.44 + 0.5366X - 227.37 \log X$	0.987
Beef (139)	−0.988 ± 0.0020	$\hat{Y} = 87.38 - 1.1684X$	1.496	$\hat{Y} = 337.88 + 0.2406X - 188.91 \log X$	1.061
Females (114)	−0.985 ± 0.0027	$\hat{Y} = 78.40 - 1.0276X$	1.145	$\hat{Y} = 401.34 + 0.6074X - 237.02 \log X$	0.992
Males (135)	−0.989 ± 0.0020	$\hat{Y} = 87.95 - 1.178X$	1.470	$\hat{Y} = 330.35 + 0.1930X - 183.06 \log X$	1.064

[a] Values in parentheses represent number of animals involved.

[b] Correlation coefficients between fat and water contents.

[c] $\hat{Y}$ = fat content (%) of whole empty body; X = water content (%) of whole empty body.

the results of recent experiments *(15, 36)* suggest that the measurement of the water content of the body of live, intact bovines is feasible, it is indicated that, should certain relationships among the components be predictable, the entire composition of the body could be resolved. The remainder of this report deals with these relationships and their extension to the prediction of the chemical composition and the energy value of the bovine body.

Relationship between the fat and water contents of the whole empty body. The relationship between the percentages of water and fat in the whole empty bovine body is shown in Figure 2. A summary of the correlation coefficients between the percentages of fat and water and of the linear and curvilinear equations for the prediction of the fat content from the water content is recorded in Table 3 for all cattle and for cattle of both of the types and sexes. These variables (fat and water contents) are highly significantly correlated inversely. As a result of earlier studies, it is assumed generally that the relationship between the percentages of fat and water is linear. Perhaps because of the larger quantity of data and the greater range in water and fat contents examined in the present study than in previous ones, the relationship between these variables was found to be significantly curvilinear. As indicated by the standard deviations from regressions shown in Table 3, the curvilinear equations express the relationship between the percentages of water and fat more accurately than the corresponding linear equations. Also, these data show that the fat content can be predicted accurately from the percentage of water in the whole empty body.

Protein and ash contents of the fat-free dry body. In an attempt to render the protein and ash contents more constant than those expressed on the fat-free basis, these components were examined on the fat-free dry basis (Table 4). The data shown in Table 4 demonstrate that the percentages of protein and ash expressed in this way are remarkably constant and that the types and sexes are not different with respect to the amounts of these components.

Highly significant correlation coefficients were found between the age of cattle and the protein (Table 5) and ash (Table 6) contents of the fat-free dry matter. This suggests that at least a large proportion of the variation in the percentages of protein and ash is associated with age. The curvilinear equations computed for the relationships between protein content and age and ash content and age do not express these relationships much more accurately than the linear

TABLE 4
Protein and ash contents of fat-free dry bodies of cattle

Cattle	Protein *(%)* (Mean ± std. dev.)	Ash *(%)* (Mean ± std. dev.)
All (251) [a]	80.26 ± 1.69	19.74 ± 1.69
Dairy (112)	80.08 ± 1.94	19.92 ± 1.94
Beef (139)	80.41 ± 1.45	19.59 ± 1.45
Females (109)	79.95 ± 1.87	20.05 ± 1.87
Males (135)	80.42 ± 1.47	19.58 ± 1.47

[a] Values in parentheses represent number of animals studied.

TABLE 5

Relationship of age of cattle to the protein content of the fat-free dry body

Cattle	Correlation coefficients[b] (± Std. error)	Regression equations[c] and std. deviations from regression			
		Linear	Sy · x	Curvilinear	Sy · x
All (251)[a]	−0.42 ± 0.052	$\hat{Y} = 80.93 - 0.00101X$	1.536	$\hat{Y} = 81.03 - 0.000964X - 0.05504 \log X$	1.530
Dairy (112)	−0.51 ± 0.069	$\hat{Y} = 80.99 - 0.00107X$	1.674	$\hat{Y} = 82.38 - 0.000557X - 0.73553 \log X$	1.613
Beef (139)	−0.21 ± 0.081	$\hat{Y} = 80.80 - 0.00078X$	1.424	$\hat{Y} = 80.03 - 0.001521X + 0.48091 \log X$	1.400
Females (109)	−0.47 ± 0.075	$\hat{Y} = 80.78 - 0.00095X$	1.656	$\hat{Y} = 81.37 - 0.000761X - 0.29619 \log X$	1.655
Males (135)	−0.23 ± 0.082	$\hat{Y} = 80.87 - 0.00087X$	1.434	$\hat{Y} = 79.96 - 0.001577X + 0.521053 \log X$	1.413

[a] Values in parentheses represent number of animals involved.

[b] Correlations between age of animal and protein content (%) of fat-free dry matter; all coefficients are highly significant.

[c] $\hat{Y}$ = protein content (%) of fat-free dry matter; X = age (days).

TABLE 6

Relationship of age of cattle to the ash content of the fat-free dry body

Cattle	Correlation coefficients[b] (± Std. error)	Regression equations[c] and std. deviations from regression			
		Linear	Sy · x	Curvilinear	Sy · x
All (251)[a]	0.42 ± 0.052	$\hat{Y} = 19.07 + 0.000101X$	1.536	$\hat{Y} = 18.97 + 0.000964X + 0.05504 \log X$	1.530
Dairy (112)	0.51 ± 0.052	$\hat{Y} = 19.01 + 0.00107X$	1.674	$\hat{Y} = 17.62 + 0.000557X + 0.73554 \log X$	1.613
Beef (139)	0.21 ± 0.081	$\hat{Y} = 19.20 + 0.00077X$	1.424	$\hat{Y} = 19.97 + 0.001521X - 0.48091 \log X$	1.400
Females (109)	0.47 ± 0.075	$\hat{Y} = 19.22 + 0.00095X$	1.656	$\hat{Y} = 18.63 + 0.000761X + 0.29619 \log X$	1.655
Males (135)	0.23 ± 0.082	$\hat{Y} = 19.13 + 0.00087X$	1.434	$\hat{Y} = 20.04 + 0.001577X - 0.52105 \log X$	1.413

[a] Values in parentheses represent number of animals involved.

[b] Correlations between age of animal and ash content (%) of fat-free dry matter; all coefficients are highly significant.

[c] $\hat{Y}$ = ash content (%) of fat-free dry matter; X = age (days)

equations (Tables 5 and 6). However, the standard deviations from regression for both forms of equations were a little less than the corresponding standard deviations from the mean protein and ash contents. These observations mean that slightly more refined estimates of the percentages of protein and ash in the fat-free, dry empty body can be predicted from the age of cattle than those which the mean percentages on the same moisture and fat basis represent.

In view of the great amount of attention given in earlier studies to the fat-free, rather than the fat-free, moisture-free, composition of animals in an attempt to reduce the composition of the body to constancy, a study was made of the relative merits of these means of expressing body composition. Obviously, only two (protein and ash) of the major body components are involved in these comparisons. The coefficients of variation (the standard deviations expressed as percentages of the respective means) on the fat-free and the fat-free dry bases, respectively, were 7.1 and 2.1% for protein and 17.8 and 8.6% for ash. The contents of protein and ash of even the fat-free body corrected for the influence of age are more variable than the mean protein and ash contents of the fat-free dry body. A decided advantage in accuracy is gained by the use of protein and ash values of the fat-free dry body corrected for age over any means of deriving the protein and ash contents on only the fat-free basis. These data, as well as others recorded in Tables 2, 4, 5, and 6, demonstrate the superior usefulness of protein and ash values expressed on the fat-free dry basis over those expressed only on the fat-free basis.

Mechanics of resolving of the composition and energy value of cattle bodies. The key to the resolution of the gross composition of the live, intact bovine lies in the accuracy with which the water content can be measured. Assuming that the percentage of water is known, the percentages of the remaining components may be derived as follows:

(a) $\hat{Y} = 355.88 + 0.355X - 202.906 \log X$, where $\hat{Y}$ = fat in whole empty body (%), and $X = H_2O$ in whole empty body (%).

(b) $100.00 - (X + \hat{Y})$ = per cent of fat-free dry matter in empty body.

(c) $P = 80.93 - 0.00101Z$, where P = protein in fat-free dry matter (%), and Z = age of animal (days).

(d) [Fat-free, dry matter (%)] $- P = A$, or $A = 19.07 + 0.00101Z$, where A = ash in fat-free dry matter (%), and P and Z are as defined in *c* above.

(e) *(P)* [Fat-free dry matter (%)] $= P_1$, where P_1 = protein in whole empty body (%), and P is defined as in *c* above.

(f) *(A)* [Fat-free dry matter (%)] $= A_1$, where A_1 = ash in whole empty body (%), and A is defined as in *d* above.

The energy value of the whole empty body then may be derived as follows:

(g) $W \cdot P_1 = P_2$, where P_2 = protein in whole empty body (g.), W = weight of whole empty body (g.), and P_1 is as defined in *e* above.

(h) $W \cdot \hat{Y} = Y_1$, where Y_1 = fat in whole empty body (g.), and $\hat{Y}$ is as defined in *a* above and W as in *g* above.

(i) $5.233^1 \cdot P_2$ = cal. stored as protein.
(j) $9.367^2 \cdot Y_1$ = cal. stored as fat.
(k) Cal. as protein + cal. as fat = total cal. in whole empty body.

Obviously, a disturbance in the normal relationship between the water and fat contents and/or the protein and ash contents could effect inaccuracies in the application of the procedure outlined. As a consequence, a comparison was made of the energy value derived from data on the body water content and age with that determined from the actual chemical composition of the bodies of cattle on which had been imposed severe experimental treatments. These comparisons, in addition to those for the control animals employed in the respective experiments, are recorded in Table 7. The caloric values of protein and fat suggested by Blaxter and Rook *(5)* were employed in these computations. These data suggest that the differences between the estimated and measured energy values of the bodies of animals fed submaintenance or maintenance rations for extended periods of time (Experiments 1 and 2), rations severely deficient in protein for approximately 400 days (Experiment 3), or a ration severely deficient in calcium for 5 years (Experiment 4) are not different from those between the estimated and measured energy values of the bodies of the control animals. The agreement between the estimated and measured energy values for the animals studied in Experiment 2 is not as good as that in the other experiments. Some of the difference may have been caused by the necessary manipulation of the body composition data in Experiment 2 in an attempt to correct these data for water losses in handling during slaughter and analysis and in correcting the analyses to the empty-body basis. Nevertheless, the data shown in Table 7 demonstrate the high degree of accuracy with which the energy value of the bovine body can be predicted from a knowledge of only the percentage of water and age of animal.

The error incurred in applying this approach to the estimation of the energy value of the body is considerably less than a casual study of the standard errors of estimate for fat and protein would indicate. An over- or under-estimation of the fat content of the whole empty body is reflected in an error of the same magnitude but of opposite direction in the estimate for the nonfat dry matter. As a consequence, the estimate for the protein content of the whole empty body is correspondingly high or low, but in a direction opposite to that of the estimate for fat content. Compensation in this manner rectifies approximately 50% of the error contributed by the estimate for fat to the total energy value of the body. It is to be expected that the error would be greater for small, thin animals than for large, fat ones.

SUMMARY

A study was made of the data published on the composition of the whole empty bodies of cattle from the standpoint of extending these data to the refinement of certain kinds of nutritional experiments. Reduction of the chemical

[1] Caloric value for bovine body protein suggested by Blaxter and Rook *(5)*.
[2] Caloric value for bovine body fat suggested by Blaxter and Rook.

TABLE 7

Measured and estimated energy value of cattle exposed to various nutritional treatments

No. of animals	Age at slaughter	Experimental conditions	Energy value		
			Measured[a]	Estimated[b]	Difference[c]
	(days)	*Experiment 1 (Ref. No. 21)*	*(Therms)*	*(Therms)*	*(%)*
1	245	Fed ad libitum	418	416	−0.6
2	254	Fed to gain ca. 1 lb. body wt/day	273	273	−0.2
3	252	Fed to gain ca. 0.7 lb. body wt/day	163	173	+6.1
4	322	Fed ad libitum	822	806	−2.0
5	326	Fed to gain ca. 1 lb. body wt/day	354	358	+1.2
6	332	Fed to gain ca. 0.7 lb. body wt/day	282	287	+1.8
7	631	Fed ad libitum	1,689	1,667	−1.3
8	796	Fed to gain ca. 1 lb. body wt/day	790	794	+0.5
9	798	Fed to gain ca. 0.7 lb. body wt/day	555	569	+2.6
10	1,200	Fed ad libitum	3,798	3,832	+0.9
11	1,215	Fed to gain ca. 1 lb. body wt/day	1,219	1,235	+1.3
12	1,228	Fed to gain ca. 0.7 lb. body wt/day	657	698	+6.2
		Experiment 2 (Ref. No. 31)			
13	476	Sub-maint. ration for 6 mo.; lost 0.5 lb. body wt/day	350	379	+8.5
14	645	Sub-maint. ration for 11 mo.; lost 0.6 lb. body wt/day	365	380	+4.2
15	506	Above-maint. ration for 6 mo.; gained 0.5 lb. body wt/day	848	872	+2.8
16	547	Maint. ration for 6 mo.; gained 0.1 lb. body wt/day	767	786	+4.1
17	650	Maint. ration for 12 mo.; gained 0.02 lb. body wt/day	462	497	+7.7
		Experiment 3 (Ref. Nos. 8 and 35)			
18	561	Steers fed a protein-deficient (ca. 4.3% total crude protein) ration for approximately 400 days	621	597	−3.9
19	585		331	320	−3.3
20	531		434	443	+2.0
21	598		374	378	+1.3
22	546	Steers fed low-protein (ca. 4.3% total protein) ration plus enough casein to provide an amount of protein equivalent to Morrison allowances during a period of about 400 days	673	677	+0.6
23	520		616	628	+2.0
24	573		719	670	−6.8
25	572		640	636	−0.5
		Experiment 4 (Ref. No. 18)			
26	4,170	Ration providing 79 g. of calcium/day for 5 yr.	978	960	−1.7
27	3,480	Ration providing 25 g. of calcium/day for 5 yr.	1,353	1,288	−4.8

[a] Measured energy value was derived from weights of protein and fat and caloric values of these substances.

[b] Estimated energy value derived from a knowledge of age of animal and percentage of water in the whole empty body.

[c] Difference (%) $= 100\left(\frac{\text{estimated} - \text{measured}}{\text{measured}}\right)$

composition of the whole empty body to the fat-free basis resulted in the following mean percentages of the major components and their standard deviations: water, 72.91 ± 2.01; protein, 21.64 ± 1.53; and ash, 5.34 ± 0.95. Despite the fact that these variations are relatively small, age was found to be highly significantly correlated with the percentages of water (−0.46), protein (0.44), and ash (0.43) in the fat-free body.

Since the results of recent studies suggest that it may be possible to determine the water content of live intact cattle, the data were examined with a view to extending interrelationships among the body components to the resolution of the chemical composition and energy value of the bovine body. It was found that the fat content *(Y)* of the whole empty body could be derived from the water content *(X)* on the same basis by use of the equation, $\hat{Y} = 355.88 + 0.355X - 202.91 \log X$. The standard deviation from regression was 1.03% fat in this population in which the mean fat content was 14.2%.

The protein and ash contents and their standard deviations in the fat-free dry body were 80.3 ± 1.69 and 19.7 ± 1.69%, respectively. There is a highly significant correlation between age of cattle and the contents of protein (−0.42) and ash (0.42) on the fat-free dry basis. A correction for age allowed more refined estimates of the amounts of these components to be made.

The application of the generalizations derived in these studies was found to provide accurate estimates of the energy value of the whole empty bodies of cattle, even under conditions in which severe experimental treatments were imposed. It is proposed that the mathematical relationships derived here be employed in conjunction with indirect measurements of the body water content to evaluate feeds and rations for cattle and to evaluate certain nutritive qualities of meat. It would appear that this approach could be applied on large scale and under practical feeding conditions.

The compositional relationships examined were not influenced by sex or type of cattle, although sufficient numbers were available for adequate comparisons to be made only between male beef and female dairy cattle.

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FIELD TRIALS WITH SEMEN CONTAINING SEVERAL COMBINATIONS OF ANTIBACTERIAL AGENTS

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Numerous papers report studies of the value of antibacterial agents in semen diluters. Different antibacterial agents, singly or in combination, have been compared *(4, 13, 21, 23, 25, 29, 34)* and different levels have been studied *(9, 11, 12, 17, 25, 26, 34)* with respect to their effect on livability of spermatozoa and bacteria counts. A number of investigations have also been reported concerning the effect of antibacterial agents upon metabolism of spermatozoa *(12, 13, 28, 31, 34)*. These studies of the action of antibacterial agents upon livability and metabolism of spermatozoa and upon bacteria contribute greatly to our knowledge of these compounds. The final test of their value, however, has to rest primarily upon their ability to aid in the production of calves by cows bred artificially. A number of direct comparisons have been made in breeding trials with different concentrations of antibacterial agents *(1, 2, 6, 17, 32)* or with different agents, singly or in combination *(3, 5, 7, 8, 10, 14, 18, 22)*. These experiments have, however, not given the final answers, in terms of fertility, concerning the most satisfactory combination or the optimum concentrations. The most informative experiment to date has been conducted by Campbell and Edwards *(14)*. They compared all possible combinations of sulfanilamide, streptomycin, and penicillin in phosphate and citrate buffers.

That the question as to the most satisfactory combination of antibacterial agents has not been settled is evidenced by information obtained from a questionnaire circulated among bull studs in the United States *(27)*. Among other questions, these stud operators were asked what combination of antibacterial agents they were employing. Of the 57 stud operators who prepared their own extender, 16 were adding sulfanilamide, penicillin, and streptomycin; seven, sulfanilamide and streptomycin; three, streptomycin only; 26, penicillin and streptomycin; and five, no antibacterial agents.

The present paper reports breeding results from two large-scale experiments conducted in studs widely scattered throughout the United States. In these two experiments different combinations and/or levels of antibacterial agents were compared. The second experiment was designed, and on a large enough scale, to give quantitative estimates *(a)* of the percentage of bulls, previously unselected in artificial breeding on the basis of fertility of their semen untreated with antibacterial agents, that are benefited by them, *(b)* of the differences in nonreturn rates between the treatments compared, and *(c)* of the value of antibacterial agents in reducing embryonic mortality. The results from these two experiments have already been reported briefly *(35)*.

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EXPERIMENTAL PROCEDURE

Experiment 1. This experiment consisted of three parts run successively. In each part two treatments were alternated with successive collections from each bull. Collections were not split. There were eight collections for each bull in Part 1, four (eight for six bulls) in Part 2, and eight in Part 3. The basic diluter consisted of one part egg yolk and one part 2.9% sodium citrate dihydrate plus sulfanilamide at a final concentration of 0.3%. When included in the diluter in Parts 1 and 2, dihydrostreptomycin sulfate was added at the rate of 500 γ and potassium penicillin G at the rate of 500 units per milliliter. In Part 1 the treatments were penicillin-streptomycin versus streptomycin only; in Part 2, no antibiotics versus streptomycin; and in Part 3, 500 units of penicillin and 500 γ of streptomycin versus 1,000 of each. Part 2 served as a control for the remainder of the experiment. Six studs cooperated in this trial. All bulls in regular service were initially included in the study. When the data were summarized and analyzed, however, data from bulls averaging less than 20 services per collection were excluded. A collection included one ejaculate or two or more ejaculates mixed together. Fertility was measured by 60- to 90-day nonreturn rates from first services performed the first day after collection. In the first part of this experiment there were 107 bulls with 856 collections and 83,816 services; in Part 2, 54 bulls, 240 collections, and 21,412 services; and in Part 3, 35 bulls, 280 collections, and 30,776 services.

Experiment 2. The general procedures were similar to Experiment 1. This experiment differed, however, in several respects. No sulfanilamide was included in the basic diluter. Four treatments were directly compared; no antibacterial agents, sulfa-streptomycin, streptomycin alone, and penicillin-streptomycin. The experimental design consisted of randomized complete blocks. For each bull 12 successive collections, none of which was split, were made over a period of 3 to 4 months. In order to minimize any time effects, one block of four collections was completed before another block was begun. Each of the four treatments was represented in each block. In the analysis of variance the estimate of experimental error was the interaction of treatments $\times$ blocks within bulls. In this experiment there were 90 bulls, 1,080 collections, and 118,875 first services in five studs.

One of the objectives of this experiment was to obtain an estimate of the percentage of bulls whose nonreturn rates were significantly benefited by the addition of antibacterial agents to the diluter. Because of the great variation in number of services per collection, the bulls were divided into three groups or strata in accordance with average number of services per collection for each bull. The ranges for each stratum were 9 to 22, 29 to 101, and 153 to 277 services per collection. After determining the within-bull, within-treatment mean square for the no-antibacterial-agent and the sulfa-streptomycin treatments combined, the least significant difference between these two treatments for individual bulls in each stratum for services per collection was calculated. In these computations t values from a one-tailed table were employed because differences in only one direction were being measured.

RESULTS AND DISCUSSION

Experiment 1. Because of stud management and other factors, all bulls were not able to complete all three parts of the experiment. For this reason, data are presented in Table 1 separately for the bulls which completed certain parts.

TABLE 1

Data from Experiment 1. Comparison of nonreturn rates between penicillin-streptomycin and streptomycin, between no antibiotics and streptomycin, and between 500 and 1,000 units each of penicillin and streptomycin (Sulfanilamide was included in all treatments)

			Part I		Part II		Part III	
Studs	Bulls		Pen.-Strep.	Strep. alone	No antibiotic	Strep. alone	500 units pen. and strep.	1,000 units pen. and strep.
(No.)	*(No.)*							
4	59	Collections *(No.)*	236	236				
		Services *(No.)*	26,891	26,856				
		Nonreturns *(%)*	66.0	66.7				
2	19	Collections *(No.)*	76	76	50	50		
		Services *(No.)*	2,192	2,347	1,858	1,890		
		Nonreturns *(%)*	67.9	70.0	63.6	69.3		
1	6	Collections *(No.)*			12	12	24	24
		Services *(No.)*			2,189	2,181	3,435	3,528
		Nonreturns *(%)*			67.6	71.4	69.2	69.0
4	29	Collections *(No.)*	116	116	58	58	116	116
		Services *(No.)*	12,638	12,892	6,640	6,654	11,974	11.839
		Nonreturns *(%)*	69.4	69.2	64.7	69.1	69.4	69.0
Grand totals and averages								
		Studs *(No.)*	6		5		4	
		Bulls *(No.)*	107		54		35	
		Collections *(No.)*	428	428	120	120	140	140
		Services *(No.)*	41,721	42,095	10,687	10,725	15,409	15,367
		Nonreturns *(%)*	67.2	67.7	65.1	69.6	69.4	69.0

In Part 1 for all data combined, there was a slight but insignificant difference in favor of streptomycin alone. In Part 2 there was a highly significant ($P < 0.001$) improvement in nonreturn rate when streptomycin was added to the diluent. In Part 3 there was a slight but insignificant difference in favor of 500 units of penicillin and of streptomycin over 1,000 units of each, indicating that 500 units are adequate.

Experiment 2. The 60- to 90-day nonreturn rates for the different treatments are given in Table 2. Fifty-seven bulls bred cows on both the first and second days after collection. The decline in nonreturn rate from first to second day was greater with penicillin-streptomycin than with the other treatments. There were 90 bulls which bred cows on the first day after collection with semen from 1,080 collections. The average nonreturn rate for semen receiving no antibacterial agent was highly significantly different ($P < 0.01$) from the mean of any other treatment *(15)*. Sulfa-streptomycin gave the highest nonreturn rate.

TABLE 2

Data from Experiment 2. Nonreturn rates with four antibacterial-agent treatments (Sulfanilamide was not included in basic extender)

Bulls	No antibacterial agents	Sulfa-strep.	Strep. alone	Pen.-strep.
(No.)				
33 Bulls used 1st day after collection only				
Services *(No.)*	18,424	18,171	18,348	18,431
Nonreturns *(%)*	64.9	71.3	69.5	68.3
57 Bulls used 1st & 2nd days after collection				
1st day				
Services *(No.)*	5,829	5,866	5,748	5,748
Nonreturns *(%)*	59.5	68.3	65.1	64.8
2nd day				
Services *(No.)*	5,279	5,320	5,734	5,542
Nonreturns *(%)*	54.0	61.8	58.3	56.3
Diff. in nonreturns *(percentage units)*	5.5	6.5	6.8	8.5
4 Bulls used 1st, 2nd, & 3rd days after collection				
1st day				
Services *(No.)*	182	176	163	182
Nonreturns *(%)*	64.3	70.4	66.2	57.7
2nd day				
Services *(No.)*	303	227	274	287
Nonreturns *(%)*	36.0	64.3	53.6	42.8
3rd day				
Services *(No.)*	128	95	110	102
Nonreturns *(%)*	34.4	64.2	35.4	29.4
90 All services 1st day after collection				
Services *(No.)*	24,253	24,037	24,096	24,179
Nonreturns *(%)*	63.6	70.6	68.5	67.5

The streptomycin treatment for all services on the first day (Table 2) was not significantly different from either the penicillin-streptomycin or the sulfa-streptomycin treatments, but the mean for sulfa-streptomycin was highly significantly different ($P < 0.01$) from the mean for penicillin-streptomycin. In these comparisons, also, there were highly significant bull × treatment and stud × treatment interactions. Analysis of variance of data from bulls with 60 or more services per collection showed little difference in results from the analysis with all data as described above.

In Table 2 with breedings by 57 bulls on the second day after collection, differences between the means for no antibacterial agents and penicillin-streptomycin and between streptomycin and penicillin-streptomycin were not significantly different. For all the other comparisons that might be made between means for breedings on the second day, the differences were significant or highly significant. Four bulls in one stud bred cows on the first, second, and third days after collection. On the third day the nonreturn rate with sulfa-streptomycin was far superior to that with the other treatments, and the nonreturn rate with penicillin-streptomycin was lower than the no-antibacterial-agent treatment. Although the numbers of services were small, there were highly significant differ-

ences ($P < 0.001$) among the four means when these third-day data were analyzed by the chi-square test in a 2×4 table.

With breedings on the first day after collection, the nonreturn rate with streptomycin alone is slightly and insignificantly higher than with penicillin-streptomycin. A similar difference occurred in Experiment 1 when sulfanilamide was included in the basic diluter. In Experiment 2 with all second-day breedings, the average nonreturn rate with streptomycin alone was two percentage units but insignificantly higher than with penicillin-streptomycin. Almquist *(7)*, Almquist and Prince *(10)*, Campbell and Edwards *(14)*, Easterbrooks *et al. (18)*, and Erb *et al. (20)* have found a higher nonreturn rate with streptomycin than with streptomycin-penicillin. Campbell and Edwards, however, had lower nonreturns with sulfa-streptomycin than with sulfa-streptomycin-penicillin. Erb and Flerchinger *(19)* with high-fertility bulls had lower nonreturn rates with penicillin than without it. Most of the data in the literature plus those presented in this paper indicate that higher nonreturn rates can be obtained with streptomycin than with penicillin in addition, whether or not sulfanilamide is also included in the basic extender. However, on the basis of viability in vitro and bacterial control *(13)* the combination of penicillin and streptomycin is superior to streptomycin. It would appear, therefore, that these two advantages of the combination of the two antibiotics are counterbalanced by some apparent detrimental effect of penicillin upon the fertilizing ability of spermatozoa. Although in vitro studies can be very useful, the data presented here serve to emphasize the necessity of breeding data to evaluate definitely the effects of different treatments upon spermatozoa.

In the study to determine how many of the 90 bulls were significantly benefited by sulfa-streptomycin as compared with no antibacterial agents (or were of low fertility without antibacterial agents), 23 (26%) were represented by differences significant at the 5% level of probability as contrasted with an expectancy of 4.5 bulls at this level of probability. One might, therefore, say that, in this particular sample, 21% (26 minus 5) of the bulls were benefited by antibacterial agents. In Table 3 are given the nonreturn rates by treatments for

TABLE 3

Data from Experiment 2. Nonreturn rates with bulls significantly benefited by sulfa-streptomycin and with other bulls

Bulls	No antibacterial agents	Sulfa-strep.	Strep. alone	Pen.-strep.
(No.)				
23 Bulls with significant differences between 1st two treatments				
Services *(No.)*	7,570	7,578	7,651	7,729
Nonreturns *(%)*	53.9	70.6	68.1	67.0
67 Other bulls				
Services *(No.)*	16,683	16,459	16,445	16,450
Nonreturns *(%)*	68.0	70.6	68.6	67.7
Diff. in nonreturns *(percentage units)*	14.1	0.0	0.5	0.7

these 23 bulls and also for the remaining bulls in this study. There is, of course, a marked difference in nonreturn rate between these two groups of bulls when no antibacterial agent was included in the diluter. Comparable differences with the other treatments are negligible, however, indicating that, with any of the three treatments which include antibacterial agents, low-fertility bulls will, on the average, maintain nonreturn rates comparable with other bulls.

The decline in nonreturn rate from 28-35 days to 60-90 days after services gives an indication of the degree of embryonic mortality. Although a number of different factors cause this decline, the chief one is embryonic mortality. Declines are given in Table 4 for individual treatments for the low-fertility bulls,

TABLE 4

Data from Experiment 2. Decline in nonreturn rates from 28-35 days to 60-90 days after service as influenced by response in nonreturn rate to antibacterial agents and by day of service (1st, 2nd, and 3rd days after collection)

		Decline in nonreturn rate *(percentage units)*			
	Bulls	No antibacterial agents	Sulfa-strep.	Strep. alone	Pen.-strep.
	(No.)				
Bulls with significant differences between 1st two treatments	23	20.2	11.4	11.8	12.9
Other bulls	67	12.1	10.9	11.5	11.4
Day of service					
First	90	14.7	11.1	11.5	11.8
Second	57	15.7	12.8	13.0	13.8
Third	4	26.1	15.8	19.1	24.4

for the other bulls in this study, and for breedings on the first, second, and third days after collection. The low-fertility bulls have an average decline of 20.2 percentage units as contrasted with a decline of 12.1 for the other bulls when no antibacterial agents were in the diluter. With the other treatments, however, the declines are similar. Here, as in the previous comparison of nonreturn rates, antibacterial agents place the low-fertility bulls on a level comparable with the others. Foote and Bratton *(24)*, Erb and Flerchinger *(19)*, and Olds *et al. (30)* also have observed a greater degree of embryonic mortality without than with antibiotics.

In Table 4 it can also be seen that embryonic mortality is consistently greatest from services on either the first, second, or third days after collection when no antibacterial agents were in the extender. This mortality becomes greater with increase in storage time of semen, in agreement with Salisbury *et al. (33)*. Although the data are based on a limited number of breedings, those on the third day after collection with diluter containing penicillin-streptomycin resulted in almost as great a rate of embryonic mortality as those with no antibacterial agents. With these third-day breedings, also, the rate of embryonic mortality was consistently lowest with sulfa-streptomycin in the diluter.

TABLE 5

Data from Experiment 2. Nonreturn rates with different treatments for different studs

Stud	Bulls		No antibacterial agents	Sulfa-strep.	Strep. alone	Pen.-strep.
	(No.)					
I	10	Services *(No.)*	5,245	5,145	5,001	5,276
		Nonreturns *(%)*	65.0	72.5	72.0	70.1
II	19	Services *(No.)*	6,017	5,928	6,054	5,909
		Nonreturns *(%)*	62.1	69.8	67.5	67.3
III	26	Services *(No.)*	8,575	8,460	8,611	8,613
		Nonreturns *(%)*	67.0	71.8	69.7	67.5
IV	27	Services *(No.)*	2,950	3,117	3,055	2,929
		Nonreturns *(%)*	59.6	68.3	63.0	64.5
V	8	Services *(No.)*	1,466	1,387	1,375	1,452
		Nonreturns *(%)*	52.3	64.2	64.2	63.8

In Table 5 are given the nonreturn rates from first-day services for the five individual studs included in this experiment. The differences between studs are highly significantly different ($P < 0.001$). In every stud the nonreturn rate with sulfa-streptomycin was superior to that with any other treatment, and fertility with no antibacterial agents was lower than with any other treatment. As stated earlier, in the analysis of variance of these data it was found that there was a significant stud $\times$ treatment interaction, indicating that the relative response to the four treatments differed from stud to stud.

SUMMARY

In the first experiment there were 107 bulls, 1,376 collections, and 136,004 services in six studs. The basic diluter was yolk-citrate-sulfanilamide. The addition of streptomycin to the basic diluter increased the 60- to 90-day nonreturn rate by 4.5 percentage units, a highly significant difference. The nonreturn rate was slightly but insignificantly higher with streptomycin than with streptomycin-penicillin. Five hundred units of penicillin and 500 γ of streptomycin gave nonreturn rates slightly and insignificantly higher than 1,000 of each.

Ninety bulls, 1,080 collections, and 118,875 services in five studs were in the second experiment. Four treatments in yolk-citrate diluter were compared: no antibacterial agents, sulfanilamide-streptomycin, streptomycin alone, and penicillin-streptomycin. A greater drop in nonreturn rate from the first to the second day after collection occurred with penicillin-streptomycin than with the other treatments. In general, the lowest nonreturn rate was with no antibacterial agents and next lowest with penicillin-streptomycin. The best results were with sulfa-streptomycin. With breedings on the second and third day after collection, the nonreturn rates for streptomycin were higher than for penicillin-streptomycin. The difference on the second day was not significant, however. The 23 bulls which were significantly benefited by sulfanilamide-streptomycin as compared with no antibacterial agents, had, with the three treatments containing antibiotics, nonreturn rates almost identical with the other bulls. Embryonic mortality, indicated by decline in nonreturn rate from 28-35 days to 60-90 days after service,

became greater with increase in length of storage of semen, was greater with no antibacterial agents, and was lowest with sulfa-streptomycin. In the analysis of variance of nonreturn rates for first-day breedings, there were significant bull × treatment and stud × treatment interactions.

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NONRETURN RATE AND EMBRYONIC MORTALITY FROM INSEMINATIONS BY BULLS WITH *VIBRIO FETUS*

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Vibrio fetus has been found to be an important factor in reducing reproductive efficiency of dairy cattle *(2, 15, 18, 19)*. The disease can be spread by artificial insemination *(1, 2, 6, 7, 10, 18)*. McEntee *et al. (11)* were unable, however, to infect 94 heifers by artificially inseminating them with extended semen containing vibrio and also sulfanilamide, penicillin, and streptomycin. Adler and Rasbech *(2)* found that seven bulls positive to vibrio had an average nonreturn rate 8.8 percentage units lower than for the average for four vibrio negative bulls. The addition of dihydrostreptomycin sulfate to the extender raised the nonreturn rate of both groups to a similar level. Studies in vitro have indicated that streptomycin is one of the most effective of the various antibacterial agents studied *(7, 11, 12, 13, 14)*. Sulfanilamide is of little value *(12)*. According to two papers *(3, 14)*, the sensitivity of vibrio to streptomycin is greater at 37° C. than at lower temperatures. However, Orthey and Gilman *(12)* found that incubation at 37° C. for 1 hour and then cooling to refrigeration temperature was more effective in destroying vibrio than continued incubation at 37° C. Three research groups *(9, 12, 14)* cultured the organism from semen in a streptomycin-containing medium after storage for 3 days at refrigeration temperature. MacPherson and Fish *(9)* also found live vibrio in semen which had been stored at –79° C. for 7 days in a medium containing streptomycin. Orthey and Gilman *(13)* recovered no live organisms after a 6-hour storage period at 5° C. In this work they followed procedures for handling semen that are practiced in artificial insemination laboratories and had concentrations of vibrio that naturally occur in undiluted semen. Their recovery of live organisms after refrigeration at 5° C. for 3 days was from semen samples with high concentrations *(12)*. Differences in concentration of the organisms and also variation among strains in sensitivity to antibiotics *(8, 13, 16)* may be responsible for the disagreement among different workers in regard to the effectiveness of antibiotics against vibrio in vitro.

Recent reports *(4, 21)* show that two types of vibrio, one believed to be pathogenic or true *V. fetus* and one believed to be saprophytic, have been isolated from the reproductive tracts of cattle. Available evidence indicates that the pathogenic, catalase-positive organism is the cause of delayed returns to service,

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abortions, and repeat breedings *(4)*. The saprophytic, catalase-negative, hydrogen sulfide-positive type is believed to be nonpathogenic, and Thouvenot and Florent *(21)* named the strain *V. bubulus*. In this paper *V. fetus* will refer only to the strain believed to be pathogenic.

Information concerning artificial insemination results in the field with bulls infected with vibrio is limited. This paper presents data obtained during the course of a large-scale field trial to compare three antibacterial-agent treatments of diluted semen with a control. Based on a limited number of tests, it was found that a number of the bulls in the experiment had been infected with vibrio, some with *V. fetus*. Data on these bulls in regard to breeding efficiency and embryonic mortality and other observations are presented.

EXPERIMENTAL PROCEDURE

The data from this large-scale trial represented 91 bulls in five studs widely scattered throughout the United States. The details of, and results from, this experiment are reported elsewhere *(22)*. The four treatments compared were: *(a)* no antibacterial agents, *(b)* 0.3% sulfanilamide and 500 γ dihydrostreptomycin sulfate per milliliter, *(c)* 500 γ of dihydrostreptomycin sulfate per milliliter, and *(d)* 500 units of potassium penicillin G and 500 γ of dihydrostreptomycin sulfate per milliliter. The basic diluter consisted of one part yolk and one part of a 2.9% sodium citrate dihydrate solution. Collections were not split. The design was that of randomized complete blocks with four treatments (and collections) in each block. Each bull was represented in the experiment by three blocks or a total of 12 collections.

After all the breedings were completed, but before complete breeding data were available, a preliminary study showed marked differences in nonreturn rates among bulls when no antibacterial agents had been included in the extender. In an attempt to explain these differences, a number of semen samples and/or preputial washings were cultured for vibrio according to the methods outlined by Bryner and Frank *(5)* and Terpstra and Eisma *(20)*. Only a limited number of samples could be studied. Samplings were continued until 7 months after the experiment comparing antibacterial agents was terminated. Twelve of the 15 vibrio cultures isolated were tested for catalase reaction.

RESULTS AND DISCUSSION

Fifteen of the 43 bulls whose semen and/or preputial washings were sampled were found to be carrying vibrio, and seven were infected with *V. fetus*. Of the ten bulls with semen samples found to be infected with vibrio, two were first detected with the first sample of semen, four with the second, two with the third, one with the fourth, and one with the fifth. Of the nine bulls whose preputial samples had vibrio, seven were detected with the first sample, one with the second, and two with the third. Vibrio was found in both semen and preputial samples from four bulls. At least five negative samples should be cultured before one can have reasonable certainty that vibrio organisms or *V. fetus* is not

present. Preputial samples have, however, been unsatisfactory for the isolation of *V. fetus*. No bull in this study had more than five semen or four preputial samples cultured. Most bulls had considerably less. In addition, the number of vibrio isolates tested for catalase ranged from one to two for each bull. For this reason, the bulls that, in this paper, are considered to be either free of vibrio or whose vibrio cultures are considered to be nonpathogenic, may not necessarily be so. Four bulls were found to carry both catalase negative and catalase positive strains.

Average nonreturn results for bulls found to be carrying *V. fetus,* those believed to be carrying nonpathogenic forms of vibrio, those carrying strains of untested pathogenicity, and those not known to be carrying vibrio, are presented in Table 1. In this table 91 bulls are represented, and only 90 in the study of nonreturn rates *(22)*. One bull with vibrio was not included in the other paper because he had only two collections per treatment instead of three. This bull averaged 242 services per collection. Of the 15 bulls found to be carrying vibrio, two had only a limited number of breedings and their data are not included in the table. The difference in average 28- to 35-day nonreturn rates between the no-antibacterial-agent and the sulfa-streptomycin treatments for the bulls with *V. fetus* was very highly significant ($P < 0.001$). Three of the seven bulls had

TABLE 1

Average fertility of bulls with and without pathogenic vibrio and with various antibacterial-agent treatments

Bulls		No agents	Sulfa-strepto-mycin	Strepto-mycin	Penicillin-strepto-mycin
(No.)					
Bulls with *V. fetus*					
7	Services *(No.)*	2,483	2,316	2,567	2,444
	Nonreturn rate *(%)*				
	28-35 days	73.4	81.5	81.1	81.0
	60-90 days	52.4	69.9	69.1	68.1
	Differences	21.0	11.6	12.0	12.9
Bulls with nonpathogenic vibrio (limited testing)					
3	Services *(No.)*	653	639	606	667
	Nonreturn rate *(%)*				
	28-35 days	75.2	79.5	80.9	78.8
	60-90 days	63.1	69.6	69.1	68.5
	Differences	12.1	9.9	11.8	10.3
Bulls with vibrio of undetermined pathogenicity					
3	Services *(No.)*	1,442	1,311	1,322	1,360
	Nonreturn rate *(%)*				
	28-35 days	75.3	79.2	80.4	76.7
	60-90 days	59.0	64.5	69.9	65.4
	Differences	16.3	14.7	10.5	11.3
Bulls not known to have vibrio					
78	Services *(No.)*	20,033	20,101	19,989	20,088
	Nonreturn rate *(%)*				
	28-35 days	79.1	81.8	79.8	79.2
	60-90 days	65.3	70.9	68.2	67.4
	Differences	13.8	10.9	11.6	11.8

differences significant at the 5% level of probability. Every one of these bulls had significant differences in 60- to 90-day nonreturn rates between these two treatments. This high incidence of bulls with significant differences is in contrast to the 21% of such bulls among the 90 studied in the large-scale trial in which these bulls had been included *(22)*. None of the three bulls with nonpathogenic vibrio, and one of the three with vibrio of undetermined pathogenicity, had a significant difference between these two treatments. When antibacterial agents were included in the diluter, the average nonreturn rates were similar for the different groups of bulls represented in the table.

The difference in nonreturn rate when measured 28 to 35 days and 60 to 90 days after service is due, at least in part, to embryonic mortality, and this difference or decline in nonreturn rate is a measure of embryonic mortality. Every one of the seven bulls with *V. fetus* had markedly greater decline when no antibacterial agents were in the diluter than when they were included. Of the other bulls with unknown or nonpathogenic types of vibrio, only one had such a decline. Furthermore, the average declines for the 78 bulls not known to have been infected with vibrio were considerably less. The results presented above indicate that semen from bulls infected with *V. fetus* causes embryonic mortality and that this loss can be effectively controlled by the inclusion of antibacterial agents in the extended semen.

TABLE 2

Influence of vibrio fetus *and antibacterial agents upon decline in 60- to 90-day nonreturn rates from first to second days after collection*

Day	Bulls	No agents		Sulfa-strep.		Strep. only		Penicillin-streptomycin	
		Serv.	NR	Serv.	NR	Serv.	NR	Serv.	NR
	(No.)	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
Bulls with *V. fetus*									
First	5	834	42.7	829	65.4	867	62.5	879	64.7
Second		726	47.0	713	56.0	809	59.1	643	56.6
Differences			−4.3		9.4		3.4		8.1
Bulls not known to be infected with vibrio									
First	52	4,995	62.3	5,037	68.8	4,881	65.6	4,869	64.8
Second		4,553	55.2	4,607	62.7	4,925	58.2	4,899	56.3
Differences			7.1		6.1		7.4		8.5

Nonreturn rates for bulls with services on both the first and second days after collection are presented in Table 2. The most striking feature of these data is that the nonreturn rate on the second day after collection was higher than on the first day (probability slightly greater than 0.05) when no antibacterial agents were included in the extended semen from the bulls carrying *V. fetus*. This is in contrast with the other treatments with these same bulls or with all treatments with the 52 other bulls, where the usual decline from first to second day occurred. A similar situation was observed by Rottensten *(17)* when he compared pregnancy rates of fertile and infertile bulls with and without

streptomycin in the extender. His breedings were performed on the day of collection and the first day after, and his increase with no antibiotic was significant. These data indicate that storage at 5° C. adversely affects *V. fetus* organisms, even without antibacterial agents. This effect may be due to attenuation, loss of vitality, and/or loss in viability of the organism. These possibilities may explain the effectiveness of antibiotics even though some workers have found that not all the organisms are killed *(9, 12, 14)*. Plastridge and Easterbrooks *(14)* and Albertsen *(3)* have suggested that the action of antibiotics against vibrio may take place at body temperature in the cervix or uterus after insemination.

SUMMARY

Data are presented which were obtained during the course of a large-scale experiment to compare three different treatments involving antibacterial agents with a control. Seven bulls were found to be infected with pathogenic vibrio *(V. fetus)* as evidenced by catalase tests. Breeding results from these bulls were compared with bulls believed to be carrying only nonpathogenic vibrio or with the other bulls in the experiment. There was a markedly greater embryonic mortality and a lower nonreturn rate among the cows bred to bulls infected with *V. fetus* when no antibacterial agents were in the extender. Results for these bulls were comparable with the others when antibacterial agents were in the extender. Streptomycin alone was as effective as treatment with streptomycin in combination with either sulfanilamide or penicillin.

Five bulls with *V. fetus* had services on the first and second days after collection. Without antibacterial agents the nonreturn rate increased from the first to the second day in contrast to the usual decline as observed with these same bulls when antibacterial agents were included in the extender or with any of the four treatments with 52 other bulls not known to have *V. fetus* and with services on the first and second days. These data suggest the possibility that *V. fetus*, while in vitro at refrigeration temperatures above freezing, tend to undergo some reduction in ability to cause infection, even in the absence of antibiotics.

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FACTORS INFLUENCING EXPERIMENTAL ERROR IN FIELD TRIALS IN ARTIFICIAL INSEMINATION

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With the expansion of artificial insemination of dairy cows in the United States and abroad there has been a steady increase in research in this field. A large part of this research has consisted of experiments wherein nonreturn rate is the criterion. Nevertheless, little information is available concerning the factors that influence experimental error in field trials. Erb *et al. (5)* made a study of standard deviations for samples varying from six to 300 services. Foote and Bratton *(6)* in an extensive study found that antibiotics decreased the among-bull variance to less than 10% of the value without antibiotics. They observed no differences, however, in within-bull variances. This paper reports studies concerning the influence upon experimental error of antibacterial agents, numbers of services per collection, infection with *Vibrio fetus*, and experimental design. A portion of these data has already been reported in abstract form *(10)*.

EXPERIMENT I. ANTIBACTERIAL AGENTS, SERVICES PER COLLECTION, AND INFECTION WITH *V. fetus*

Experimental Procedure.

The data upon which this study was based were from an experiment reported elsewhere *(11)*. Four treatments were compared: no antibacterial agents, sulfanilamide-streptomycin, streptomycin, and penicillin-streptomycin. The experimental design consisted of randomized complete blocks. For each bull 12 successive collections, none of which was split, were made over a period of 3 to 4 months. In order to minimize any time effects, one block of four collections was completed before another block was begun. Each of the four treatments was represented in each block. The variance among collections treated alike (within bulls within treatments, or within subclasses) was the measure of experimental error.

In the experiment were 90 bulls of the Aberdeen Angus, Brown Swiss, Guernsey, Hereford, Holstein-Friesian, Jersey, and Shorthorn breeds. These bulls were in service in five studs scattered widely throughout the United States. The number of cows bred to the individual bulls varied greatly, although the numbers of services were similar for the bulls within each breed within each stud. For this reason, the data for the bulls within each breed in each stud were grouped together, and these groups were combined in different strata depending upon the

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average number of services per collection in each group. The average number of services per collection for the different strata and the breeds contained in each were as follows: 16, Aberdeen Angus, Brown Swiss, Hereford, and Jersey; 42, Aberdeen Angus, Guernsey, Jersey, and Shorthorn; 80, Guernsey and Jersey; 168, Guernsey; and 256, Holstein. With each of these strata the error variance was computed separately for each antibacterial-agent treatment. The probability levels for the differences among error variances were computed by means of Bartlett's tests of homogeneity *(7)*. The data included 96,565 services from 1,080 collections. Throughout this paper breeding efficiency is measured by 60- to 90-day nonreturn rates from first services performed the first day after collection.

When the inseminations were completed but before all the breeding results were available, a preliminary check showed great differences among bulls in the degree to which antibacterial agents benefited them. In an attempt to explain these differences, semen samples and/or preputial washings from a number of bulls were cultured and examined under a microscope for vibrio. Only a limited number could be examined. The method was that described by Terpstra and Eisma *(8)* and by Bryner and Frank *(3)*. Since only one to five samples per bull tested could be checked for vibrio, the bulls with samples in which no organisms were found were not necessarily uninfected. Furthermore, some of the bulls which were not tested may also have been harboring the disease. Vibrio cultures from 12 of the bulls were subjected to the catalase test *(2)*. There is good evidence that biochemical tests differentiate between pathogenic and nonpathogenic strains *(2, 9)* and that only the pathogenic strain produces delayed returns to service, abortions, and repeat breedings and is true *V. fetus (3, 13)*.

Results.

In Table 1 are presented the error variances for each treatment (and for the combination of the three treatments with antibacterial agents) for each stratum. In general, as the average number of services per collection increased, error

TABLE 1

Data from Experiment I. Error variance with different antibacterial-agent treatments and different numbers of services

Services per collection (sample) *(No.)*						
Range	9-22	29-56	60-101	147-183	224-277	9-277
Average	16	42	80	168	256	89
Bulls *(No.)*	27	20	18	12	13	90
Collections *(No.)*	324	240	216	144	156	1,080
Services *(No.)*	5,097	10,098	17,168	24,183	40,019	96,565
Nonreturn rates *(%)*	67.3	65.8	64.7	68.1	68.8	67.5
Treatments:						
No antibact. agents	185.0	76.4	89.2	77.5	71.6	111.0
Sulfa-streptomycin	227.9	69.1	43.9	31.2	15.6	98.9
Streptomycin	201.0	105.2	41.3	44.0	23.1	101.1
Pen.-streptomycin	256.7	109.3	49.9	33.6	53.2	123.4
Three with antibacterial agents	228.5	94.5	45.0	36.3	30.6	107.8
Probabilities among treatment variances	0.4	0.16	0.01	0.02	< 0.0001	< 0.05

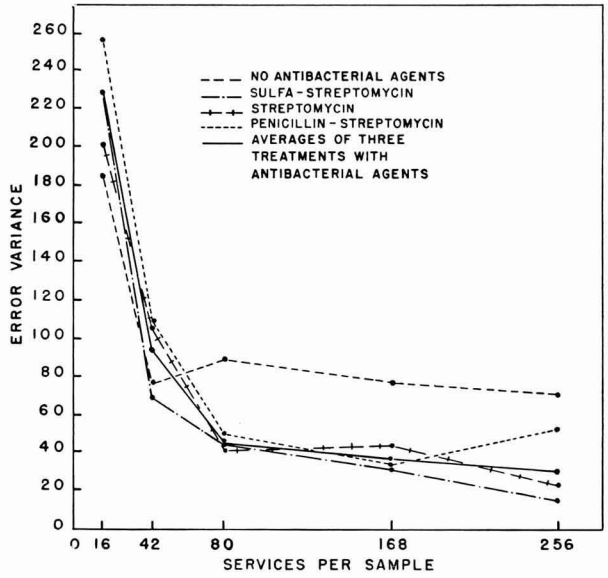

FIG. 1. Relation between error variance and number of services per sample.

variance decreased, and the ability to measure heterogeneity among error variances increased, as indicated by lower probability values. In Figure 1 the relationship between error variances for the treatments, individually and combined, and number of services per collection is shown. It can be seen that error variance declined rapidly with increase in numbers until approximately 80 services per collection were attained. As numbers were increased above this level the decline in error variance was comparatively small in proportion to the increase in number of services.

In Table 2 are presented error variances for the seven bulls whose vibrio cultures were positive to the catalase test and for the six bulls with vibrio. The

TABLE 2

Data from Experiment I. Effect of antibacterial-agent treatments upon error variance with bulls with vibrio

Bull groups	Bulls with *V. fetus*	Other bulls with vibrio
Bulls *(No.)*	7	6
Collections *(No.)*	84	68
Services *(No.)*	9,810	8,000
Av. services per collection (sample) *(No.)*		
Averages	117	118
Ranges	39-277	47-265
Treatments		
No antibacterial agents	61.2	52.2
Sulfa-streptomycin	36.8	58.3
Streptomycin	133.8	44.8
Pen.-streptomycin	50.6	15.4
Three with antibacterial agents	73.7	39.5
Probabilities	0.02	0.02

error variance for all treatments for the seven bulls with *V. fetus* was somewhat higher than that for the corresponding value in Figure 1 where the number of services was similar. The comparable figure for the six other bulls with vibrio was actually lower than would be expected on the basis of Figure 1.

EXPERIMENT II. STUDY OF EXPERIMENTAL DESIGN

Experimental Procedure.

In a study to be reported later, nonreturn rate data were compiled from two studs breeding cows over a large portion of the United States east of the Mississippi River. Semen was shipped from these studs on alternate days to the same area. The data were computed by bulls by states by days. A portion of these data covering the months of January, February, and March, 1952, was compiled for the study to be reported below. Services and nonreturns for two customer groups were obtained from individual collections. Each customer group consisted of one or more states, and the paired groups or half-collections had a similar number of services. By thus pairing as many single states or combinations of states as possible a considerable range in services per half-collection resulted. The data from bulls within each stud within each breed for each month were grouped together and stratified according to the average number of services per half-collection for each group. The strata were the same as the three lowest in Experiment I. Relative efficiency was calculated as outlined by Cochran and Cox *(4)*. This gives a measure of the relative amount of replication required to give the same degree of precision; an experimental design with a relative efficiency of 50% would require twice as much replication as one with 100%.

Since the semen received no experimental treatments, this experiment can be considered a uniformity trial. Three different designs were superimposed upon the data as follows: *(a)* Split-collection design with half-collections. The nonreturn percentages for the two customer groups within each collection were considered as paired values. Thus, each unit served as one half of a split collection. Experimental error was the variance for the interaction of collection × customer groups within bulls. *(b)* Whole-collection design with half-collections. The nonreturn percentages for the two customer groups within each collection were considered independent. Thus each unit was a half-collection but was treated in the analysis as a whole collection. Experimental error was the variance for the half-collections within bulls within customer groups. *(c)* Whole-collection design with whole collections. The nonreturn percentages were based on the totals of the services and nonreturns for both customer groups within each collection. Thus, each unit averaged twice as many services as in *a* or *b* and was considered to be a whole collection in the analysis. Experimental error was the variance among whole collections within bulls.

The semen was extended in yolk-citrate in proportions of one part yolk and one part of 2.9% sodium citrate dihydrate. The extender included 0.3% sulfanilamide and 500 γ of dihydrostreptomycin sulfate and 500 units potassium penicillin G. The analyses were based upon 25,042 services from 349 collections

TABLE 3

Data from Experiment II. Error variance with different experimental designs—data from two studs with collections split two ways

Bulls *(No.)*	17	13	10
Collections *(No.)*	158	143	50
Services *(No.)*	5,417	11,594	8,031
(a) Split-collection design with half-collections			
Av. services per unit *(No.)*	17	39	80
Samples *(No.)*	316	296	100
Error degrees of freedom	137	130	43
Error variance	276.4	88.9	41.2
Relative efficiency *(%)*	74	85	213
(b) Whole-collection design with half-collections			
Av. services per unit *(No.)*	17	39	80
Samples *(No.)*	316	296	100
Error degrees of freedom	192	188	66
Error variance	231.3	86.0	62.6
Relative efficiency *(%)*	88	88	140
(c) Whole-collection design with whole collections			
Av. services per unit *(No.)*	34	78	161
Samples *(No.)*	158	148	50
Error degrees of freedom	96	94	33
Error variance	101.9	38.0	43.9
Relative efficiency *(%)*	100	100	100

from 40 bulls of the Guernsey, Jersey, and Holstein-Friesian breeds. Since some of the same data by states appeared more than once as a result of the different paired groupings, the actual number of services and collections was somewhat less than the above figure.

Results.

The data for this study are presented in Table 3. With an average of 17 services per experimental unit, the split collection experiment is somewhat less efficient than where collections are not split. With an average of 39 services per collection the difference in efficiency was slight. In both instances the relative efficiency was increased when the data from the two customer groups were combined and each unit had double the number of services. When each experimental unit averaged 80 services, the relative precision of the three designs is not the same as above. The split-collection design was much more efficient than the whole-collection design whether the average number of services per unit was 80 or 161. Throughout these comparisons where the average number of services per unit was the same, there were more degrees of freedom for error with the whole-collection than with the split-collection design.

EXPERIMENT III. STUDY OF EXPERIMENTAL DESIGN

Experimental Procedure.

For this uniformity trial, data were obtained from Holstein and Guernsey bulls in regular service in a bull stud during a period when the semen was not subjected to any experimental treatments. The customers were divided into four groups breeding approximately the same number of cows, and nonreturn rates were obtained for each collection for each customer group. In the experiment were 9,299 services and 57 collections from 15 bulls. Breedings were per-

formed during the months of December, 1951, and January, 1952. It was planned to have each bull represented by four collections. Because of the holidays there was, however, an extremely small number of services from some collections, which were not included in the study. As a consequence, three of the bulls were represented by only three collections. The semen extender was prepared in the same manner as that in Experiment II.

Since the numbers of services for the Guernsey bulls were much smaller than for the Holsteins, the data for the breeds were analyzed separately. The designs superimposed on the data were as follows: *(a)* Split-collection design with quarter-collections. Nonreturn percentages were calculated for the four customer groups within each collection. In the analysis each collection was considered as being split four ways. Experimental error was the interaction of customer group × collections within bulls. *(b)* Whole-collection design with quarter-collections. The quarter-collections within each collection were considered independent. Experimental error was the variance among quarter-collections within bulls within customer groups. *(c)* Split-collection design with half-collections. The services and nonreturns for the first and second and for the third and fourth customer groups were combined, and nonreturn percentages were calculated. Each unit consisted of a half-collection and was based upon twice as many services as in *a* and *b*. The data were analyzed as split collections as in *a*. *(d)* Whole-collection design with half-collections. The half-collections within each collection were considered independent. Experimental error was the variance among half-collections within bulls within each of the two customer groups. *(e)* Whole-collection design with whole collections. The nonreturn percentages were based upon the totals of the services and nonreturns for all customer groups within each collection. Each unit, thus, had four times as many services as in *a* and *b* and twice as many as in *c* and *d*. Experimental error was the variance among collections within bulls.

Results.

In Table 4 are presented the results of this study. In general, the picture is similar to that in Experiment II. In the first column of data (Guernseys) with 16 or 31 services per experimental unit, or when the data for each collection are combined to give 62 services per experimental unit, the design based upon within-subclass error variance is the most efficient. With the Holsteins, which have greater numbers of services per unit, the split-collection design was more efficient. However, the degrees of freedom for error variance is greater for the whole-collection than for the split-collection design.

DISCUSSION AND CONCLUSIONS

The data presented above demonstrate that experimental error in artificial insemination experiments in the field may be influenced by various factors:

A. *Antibacterial agents.* The variance among nonreturn percentages is influenced greatly by these compounds. With an average of 80 or more services per

TABLE 4

Data from Experiment III. Error variance with different experimental designs—data from uniformity trial with collections split four ways

	Guernseys	Holsteins
Bulls *(No.)*	7	8
Collections *(No.)*	27	30
Services *(No.)*	1,685	7,614
(a) Split-collection design with quarter-collections		
Av. services per unit *(No.)*	16	63
Samples *(No.)*	108	120
Error degrees of freedom *(No.)*	78	87
Error variance	154.9	42.7
Relative efficiency *(%)*	44	172
(b) Whole-collection design with quarter-collections		
Av. services per unit *(No.)*	16	63
Samples *(No.)*	108	120
Error degrees of freedom *(No.)*	80	88
Error variance	134.0	49.6
Relative efficiency *(%)*	51	152
(c) Split-collection design with half-collections		
Av. services per unit *(No.)*	31	127
Samples *(No.)*	54	60
Error degrees of freedom *(No.)*	26	29
Error variance	91.2	25.4
Relative efficiency *(%)*	37	148
(d) Whole-collection design with half-collections		
Av. services per unit *(No.)*	31	127
Samples *(No.)*	54	60
Error degrees of freedom *(No.)*	40	44
Error variance	64.7	32.2
Relative efficiency *(%)*	53	117
(e) Whole-collection design with whole-collections		
Av. services per unit *(No.)*	62	254
Samples *(No.)*	27	30
Error degrees of freedom *(No.)*	20	22
Error variance	17.0	18.8
Relative efficiency *(%)*	100	100

collection, the error variance was largest when there was no antibacterial agent in the diluter. Differences among the variances for different treatments were below the 0.0001 level of probability when the average number of services per collection was 256. In this stratum, where precision is greatest, the magnitude of these error variances for individual treatments is in reverse order to the nonreturn rate means *(11)*. This fact supports the conclusion drawn in the paper just cited that the combination of sulfanilamide and streptomycin is most effective of the treatments studied.

B. *Number of services per experimental unit.* The curves in Figure 1, representing each and the average of the three treatments with antibacterial agents, agree closely with expected values based upon the confidence limits given by Snedecor in Table 1.1 *(7)*. The data in Tables 3 and 4 tend to follow the general trend of the line representing averages of the three antibacterial-agent treatments on the graph. Furthermore, in another study involving 236,296 services performed by 137 field technicians over a period of 1 year *(12)*, the trend of this line was reproduced very closely. In this unpublished study each experimental unit was a technician-month, and the error term was the within-technician within-year variance. These various facts indicate that the relationship

between numbers of services per unit and experimental error in nonreturn rates is due in most part to random variation and approaches theoretical expectations. Erb *et al. (5)*, on the other hand, observed a leveling off of standard deviations with more than 30 services per sample. Their observations were made on subsamples selected from collections within a narrow range of fertility. Data presented in Table 1 demonstrate the fact that, when number of services per sample is small, the experimental error is large and treatment effects must be large to be significant. With small numbers of services, such as 9-22, there were no significant differences among the error variances. With 224-277 services the range of the error variances was less than with 9-22 services. With greater precision, however, it was possible to detect differences in heterogeneity with a probability of < 0.0001.

C. *Disease.* As indicated above, seven bulls in Table 2 with *V. fetus* had, on the average, higher error variances than would be expected from Table 1. The error variance was highest with streptomycin alone. This large variance was due to the extremely high value for one bull. For this reason it is doubtful if the variance for streptomycin for these seven bulls is representative of the population. It is known that, when vibrio (or trichomonad) organisms are cultured from different ejaculates or collections of individual bulls, the number of organisms present vary greatly. It could, therefore, be hypothesized that there would be greater variation in fertility from collection to collection within bulls with pathogenic organisms than within disease-free bulls. This possibility is not definitely borne out by the data in Table 2. The data in Table 1 for strata with 60 or more services per sample do, however, support this hypothesis. The difference between the error variance for no antibacterial agents and for sulfastreptomycin for each of the two strata with 60-101 and 147-183 services per collection is significant. The difference for the stratum with 224-277 services per collection is highly significant ($P < 0.002$).

D. *Experimental design.* According to the data in Tables 3 and 4, the most efficient design depends upon the number of services per collection. If the number averages much less than 160, collections should not be split, for if split the number of services per experimental unit would be less than 80. Treatments should then be randomized among successive collections within individual bulls. One plan would be that followed in the experiment from which the data in Table 1 were derived. Another advantage of not splitting collections is that the breeding results are obtainable from routine data; no special computations, such as required for split collections, would be needed. With more than 160 services per collection the split-collection technique can be used. The number of experimental units that can be made from each collection should, however, be such that the number of services per unit will not be much below 80. The amount of information obtained will be greater than would be derived from the same number of collections not split. If each collection could be split only two ways and it is desired to compare more than two treatments, incomplete block designs such as those outlined by Bose *et al. (1)* could be employed.

More work is needed to establish more precisely than has been done in this paper the relative precision of different experimental designs. Considerable improvement in efficiency undoubtedly could be obtained in many experiments by using the many designs and techniques that have been developed by statisticians.

SUMMARY

In Experiment I, involving 96,565 services from 1,080 collections from 90 bulls, error variances with four antibacterial-agent treatments were compared. The results were as follows: *(a)* With an average of 60 or more services per collection (not split) there were significant differences among variances for collections within bulls within treatments (error) for the four antibacterial-agent treatments studied, with the lowest being with the sulfa-streptomycin treatment and the highest with no antibacterial agents. *(b)* With smaller numbers of services per collection differences in heterogeneity of the error variances could not be detected. *(c)* Error variance decreased rapidly as numbers of services were increased to 80. Above this number the decrease in error variance was slight in proportion to the increase in numbers. *(d)* There was a little evidence suggesting that there was more variation among collections within bulls known to be infected with pathogenic vibrio than other bulls.

In Experiments II and III, comprising a total of 34,341 services, a study of the effect of experimental design upon relative efficiency indicated that, if collections average more than 160 services, they can be split into as many units as desired as long as each unit represents an average of 80 or more services. If collections average much less than 160 services, they should not be split and incomplete block or randomized complete block designs should be employed.

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THE RESPONSE OF DAIRY CALVES TO AUREOMYCIN FED WITH A LIBERAL MILK AND GRAIN RATION

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Numerous investigations have demonstrated that aureomycin included in the diet of the calf promotes a faster growth rate than nonsupplemented rations *(1, 2, 4, 7, 8)*. Most of these reports have shown that the aureomycin-supplemented calves also consumed more grain and/or hay than comparable control calves, and in some experiments there has been an improved efficiency of TDN utilization. Under usual feeding regimes calves are fed milk equivalent to 10% of body weight and starter to a maximum of 4 lb. per day. Since calves do not eat much starter until weaned, this early regime provides much less food than normally would be consumed by suckling milk ad lib. Milk allowances up to 20% of body weight are consumed easily by healthy young calves *(5, 9)*. This experiment was conducted to determine how much growth response could be secured with and without aureomycin when calves were fed very liberally on milk and a milk replacement mix and allowed a liberal starter ration along with good quality hay free choice.

EXPERIMENTAL PROCEDURE

Jersey and Holstein-Friesian calves in the University of Tennessee herds at Knoxville and Columbia were divided into two groups by alternately assigning normal calves of each breed and sex to the groups as they were born from February to December, 1952. The calves were trained to drink from a bucket and were fed three times per day for the first 10 days. Normal sized Holsteins were allowed all of the milk they would consume up to 6 lb. per feed and Jerseys were allowed up to 3.3 lb. per feed. From the seventh to the tenth day the milk was gradually replaced with a reconstituted milk replacement made by mixing 8.5 parts warm water and 1.5 parts of dry powder. The powder mix was nonfat dry milk solids, 60%; dried whey, 20%; distillers dried solubles, 10%; dextrose, 7%; vitamin A and D concentrate, 0.5%; iodized salt, 0.5%; and a mineral mix providing calcium, phosphorus, magnesium, iron, copper, manganese, and cobalt, 2.0%.

A pelleted 18% crude protein calf starter and a good quality of alfalfa and mixed grass hay were fed ad lib. after the third day. Holsteins were limited to 7 lb. and Jerseys to 5 lb. of starter daily. From the 11th to the 30th day the reconstituted "milk" was fed twice daily, allowing up to 20 lb. for Holsteins and 12 lb. for Jerseys daily, which amounts approximated 15 to 20% of body weight. Six Holsteins which weighed 60 lb. or less at birth were fed milk at the Jersey allowance. From the 31st to 50th day the milk was gradually decreased

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to zero, and an effort was made to get the calves to eat more starter. The calves were kept in individual pens with free access to drinking water at all times. Feed consumption was recorded daily for the calves at Knoxville. Calf weights and heights were determined at weekly intervals until 120 days, when the experiment was terminated.

One group was fed as outlined above without supplement. The other group was given aureomycin in the milk replacement and starter. Aureomycin in Aurofac D or Aureomycin TF was stirred into the milk at the rate of 50 mg. per feed for Holsteins and 30 mg. for Jerseys. The milk replacement contained 50 mg. of aureomycin per pound and the calf starter contained 75 mg. aureomycin per pound.

RESULTS

A total of 33 Jerseys and 39 Holsteins were born alive during the experiment and were potentially usable. Two Holsteins and five Jerseys were eliminated from the groups in the first few days because of their weakened or abnormal physical condition, and two Holsteins were eliminated later for refusal to eat or drink the experimental rations satisfactorily. Of the 35 Holsteins and 28 Jerseys used in the final comparisons (see Table 1), two Holsteins, one from each group, died from pneumonia at 112 and 120 days, respectively; and four Jerseys, one control and three aureomycin-fed, died between weaning and 120 days from pneumonia or undetected causes. Deaths were not reduced by the aureomycin feeding in this experiment. Considerable looseness of stools which might be termed diarrhea or scours occurred in both groups during the milk

TABLE 1

Summary of weight and height gains, feed consumption and efficiency of Holstein and Jersey calves comparing liberal milk and starter feeding with and without aureomycin

	Holsteins		Jerseys	
	Control	Aureomycin	Control	Aureomycin
Bull calves *(No.)*	11	10	4	5
Heifer calves *(No.)*	6	8	10	9
Beginning weight *(lb.)*	88.1 ± 16.9[a]	88.3 ± 15.0	54.8 ± 7.9	53.4 ± 5.7
Weight gains:				
1 to 29 days *(lb.)*	34.8 ± 12.2	38.8 ± 13.8	21.8 ± 10.8	27.4 ± 14.5
1 to 50 days *(lb.)*	51.2 ± 17.2	56.4 ± 19.5	30.7 ± 17.4	41.9 ± 19.0
50 to 120 days *(lb.)*	112.2 ± 41.2	128.2 ± 31.4	82.9 ± 28.2	84.4 ± 20.0
1 to 120 days *(lb.)*	163.4 ± 39.8	184.6 ± 27.0	113.6 ± 24.5	126.3 ± 19.8
119-day daily av. *(lb.)*	1.37	1.55	.96	1.06
Ht. increase, 1 to 120 days *(in.)*	7.14 ± 1.4	7.21 ± 1.4	6.75 ± 1.2	7.36 ± 1.4
Feed consumed, 119 days:				
Milk *(lb.)*	710	715	456	465
Starter *(lb.)*	389	447	313	327
Hay *(lb.)*	87	100	83	83
Estimated TDN *(lb.)*[b]	416.1	466.0	326.8	338.4
TDN per lb. gain *(lb.)*	2.55	2.52	2.88	2.68

[a] Mean ± standard deviations.

[b] TDN estimations assumed 75% TDN from starter, 45% TDN from hay, and 12% TDN from milk consumed.

feeding period. This was probably due to the high whey and lactose content of the "milk," and there was no indication that aureomycin prevented or alleviated the condition.

The growth results are presented separately according to breeds and treatments. The average growth (weight gain) curves are presented in Figure 1. These show that Holstein calves on both rations gained at about the same rate through the seventh week, while milk was fed, but that thereafter the aureomycin group steadily gained faster. The control Jersey calves, however, lagged behind the aureomycin group until after milk-feeding ceased, and then both groups gained at about the same rate. In both groups in both breeds the rate

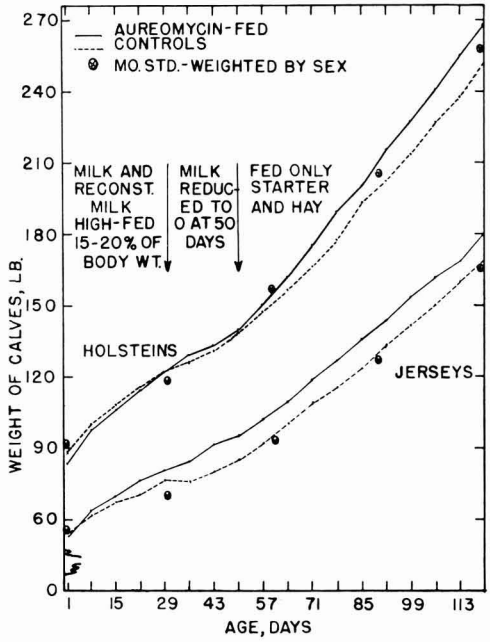

FIG. 1. Average weekly weights of 28 Jersey and 35 Holstein calves fed on a liberal early milk diet with and without aureomycin to 120 days of age.

of gain was above normal during the liberal milk feeding, then it declined during the 3 weeks of gradual milk reduction, and it returned to normal or above after stopping the milk. Many of the calves did not consume starter satisfactorily as long as they were fed some milk. The aureomycin group consumed more starter during the milk reduction period and the week following weaning and maintained nearly a normal rate of gain during the changeover period.

The average weight gains by periods, height gains, and feed consumption are listed in Table 1. Under the system of random assignment to groups (by alterna-

tion) it happened that the initial weights of the control group averaged higher than those of the supplemented group in both breeds. In spite of this initial handicap the aureomycin groups outgained the controls in the first 28-day period of high milk feeding by 4.0 lb. for Holsteins and 5.6 lb. for Jerseys. The average differences in gains during the entire 49-day milk-feeding period were 5.2 lb. for Holsteins and 11.2 lb. for Jerseys. Because of the wide variation in gains, as shown by the large standard deviations in Table 1, the average differences due to rations were not statistically significant for any of the periods. The aureomycin groups consumed slightly more milk, starter, and hay than the control groups, which resulted in practically the same feed efficiency per pound of gain.

DISCUSSION

These results have not been interpreted as either for or against aureomycin-feeding along with liberal milk rations. The wide variations observed were undoubtedly due to the year-long period of the experiment, including all normal calves as they were available at the various seasons of the year. This is the situation which would exist in dairy herds, but it is somewhat different from most previous experiments with aureomycin in calf feeds. Under these conditions it is very likely that many of the control calves in individual pens were not exposed to conditions against which aureomycin would have been effective *(1)*; thus they grew just as well as the best of the aureomycin-fed group. Other control calves were definitely subnormal at some time during the experiment, and so, in fact, were several of the aureomycin group. Aureomycin might have improved some of the control calves, but it obviously was not effective against all infectious conditions or none of the aureomycin group would have died. The average differences, although not statistically significant, indicate that aureomycin may slightly improve the growth rate and feed consumption of some calves while they are being fed liberally with milk, as well as after weaning. These observations are supported in part by similar small average increases in growth rate of calves fed heavily on milk for veal production, as reported by Johnson *et al. (6)* and Gardner *et al. (3)* when aureomycin was fed with the milk. In a later experiment, Hopper *et al. (5)* found that the growth stimulus of aureomycin for heavily fed veal calves was very slight. It should, therefore, not be expected that the response to aureomycin feeding would be as great when calves are fed large quantities of milk as when milk is limited. The smaller differences in gains in this experiment, 11.2% for Jerseys and 12.9% for Holsteins, as compared with previous experiments on limited milk feeding plans *(1, 7, 8)* in which increased gains of about 25% were obtained, were probably due in large part to the higher level of milk feeding of this experiment, which allowed the control calves to make above normal gains in spite of a low appetite for grain and hay. If whole milk or its equivalent had been fed in place of the low-fat milk replacement, as in the Illinois experiments *(3, 5, 6)*, the differences due to aureomycin feeding might have been even less than those observed.

SUMMARY

The growth-promoting effect of aureomycin added to a calf ration which provided 15 to 20% of body weight daily of milk and a milk replacement mixture for the first 30 days was determined with 28 Jersey and 35 Holstein calves. The average gains of the aureomycin-fed calves exceeded those of the control calves during the milk feeding period as well as after weaning, but the differences were not statistically significant because of the wide variations in gains within each group. These results indicate that on a liberal milk feeding regime for calves growth stimulation due to aureomycin will not be attained consistently.

ACKNOWLEDGMENTS

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TECHNICAL NOTES

ELIMINATION OF 9-DECENOIC ACID AS A PRECURSOR OF δ-DECALACTONE IN MILK FAT[1]

During the early storage period of certain fat-containing dairy products, a flavor defect described as "coconut-like" develops. The flavor compound has been isolated from butter oil and identified as δ-decalactone *(6)*. Indications are that the flavor precursor is unique to milk fat since refined animal and vegetable fats do not develop a coconut-like flavor *(4)*. Unrefined fats have not been thoroughly investigated in this respect. Grün and Wirth *(3)* observed that 9-decenoic, a mono-unsaturated, ten carbon fatty acid of milk fat, develops the odor of decalactone when exposed to air for several months. In view of the fact that 9-decenoic acid does not occur in any other common animal or vegetable fat *(5)*, the suggestion was made that this acid would be a logical source of the coconut-like flavor of milk fat *(7)*. Therefore, a study was made to determine if 9-decenoic acid may serve as the precursor of δ-decalactone.

Nine-decenoic acid was synthesized by a Barbier-Wieland degradation of 10-hendecenoic acid. Two moles of phenyl magnesium bromide were reacted with one mole of ethyl 10-hendecenoate. The tertiary alcohol thus formed was brominated to protect the double bond, and then dehydration was accomplished by agitating the brominated alcohol for 4 hours at 60° C. in the presence of 1,400 ml. of methanoic acid. Benzene extraction yielded 450 g. of hydrocarbon residue which were then oxidized to dibromodecanoic acid by dissolving the hydrocarbon in ethanoic acid and adding, dropwise, 280 g. of chromium trioxide dissolved in 300 ml. of water. The benzene extract from this reaction mixture was shaken with dilute NaOH solution, and after the aqueous alkaline layer had been acidified the dibromodecanoic acid was extracted with benzene. Debromination was accomplished by refluxing with a mixture of 3 l. of methanol, 300 g. of zinc dust, and 5 ml. of HCl. The decenoic acid fraction obtained from this reaction mixture was distilled several times under reduced pressure. Forty g. of 9-decenoic acid were obtained boiling at 130-135° C. (1-3 mm.).

Properties of 9-decenoic acid	*Obtained*	*Literature or calculated*
Molecular weight (by titration)	169.7	170.2
Iodine No. (Wijs)	148.4	149.2
Refractive Index n_D^{20}	1.4488	1.4488 (3) 1.4462 (1)

Oxidation with chromic acid yielded azelaic acid (m.p., 106° C., neutralization equivalent, 94). Additional proof that the decenoic acid was terminally unsaturated was obtained by infrared analysis. The strong absorption exhibited at 10.12 and 11.02 μ can logically be attributed to vinyl unsaturation *(2)*.

Experiments with 9-decenoic acid. A series of experiments was conducted to ascertain if 9-decenoic acid is the precursor of δ-decalactone. This was approached by attempting to generate the coconut-like odor of the lactone by: leaving a small sample of 9-decenoic acid exposed to air at room temperature; refluxing the acid with water; refluxing the acid in an aqueous alkaline solution followed by acidification; adding copper to a water solution of the acid; autoclaving butter oil and hydrogenated coconut fat, each containing 1% added 9-decenoic acid; and refluxing 9-decenoic acid with 70% sulfuric acid. The samples were evaluated periodically over a period of eight months duration. Only the H_2SO_4 treated sample produced the typical coconut-like odor. The other treated samples were described as soapy, sweet, or metallic. None of these samples, with the exception of the H_2SO_4 treated sample, emitted an odor character that was, in any way, suggestive of decalactones. The results of this study indicate that 9-decenoic acid is not directly involved in the formation of δ-decalactone in fat-containing dairy products. The factors known to accelerate the development of the coconut-like defect in milk fat, that is, heat, storage, and moisture, did not generate a coconut-like character from 9-decenoic acid. Grün and Wirth's *(3)* observation that 9-decenoic acid develops the odor of decalactone when exposed to air for several months could not be confirmed.

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APPARENT SELECTIVE LIBERATION OF BUTYRIC ACID FROM MILK FAT BY THE ACTION OF VARIOUS LIPASE SYSTEMS[1]

Wilcox *et al. (2)* have shown recently that cell-free microbial lipases differ in their ability to release volatile fatty acids from milk fat. Evidence of apparent selective hydrolysis of butyric acid from milk fat by various non-microbial lipase systems is presented in this report.

Pancreatic lipase (General Biochemical Company), milk lipase (freeze-dried raw milk powder), and glandular lipases (prepared by Dairyland Food Laboratories) from different animal sources were investigated. Cream homogenized at 2,500 p.s.i. was used as the substrate. The enzyme systems were prepared to 0.2 *M* pH 6.6 phosphate buffer. The concentration of enzyme was adjusted so that the reaction rate was linear in respect to enzyme concentration. The enzyme preparation and substrate were combined so that the fat content of the reaction mixture was 10%. The samples were reacted with gentle shaking at 37° C. for 5 hours. The total free fatty acids and free butyric acids were measured after 1, 3, and 5 hours of incubation *(1)*. The results were calculated as the per cent butyric acid present in the total free fatty acid mixture (based on a microequivalent basis).

The relative per cent butyric acid varied between 1 and 3 hours but was the same at 3 hours as at 5 hours of reaction.

The data in Table 1 show the acid degree increase resulting from lipolysis and the per cent butyric acid present in the total free acids from five different enzyme preparations. The results are based on ten separate trials and are presented as the average value, range, and standard deviation from the mean.

The data presented in Table 1 for five different enzyme systems reveal marked differences in the relative concentration of butyric acid hydrolyzed from milk fat. Although butyric acid is present in about 10 *M* % in unhydrolyzed milk fat, several preparations (the glandular lipases) preferentially hydrolyzed butyric acid from the fat so that it was four times this concentration in the free acid fraction. Milk lipase showed slight selectivity based on the higher concentration of this acid in the free acid position, but pancreatic lipase exhibited the opposite effect.

The importance of lipase source in selecting a system for the selective liberation of butyric acid, such as is desired in the manufacture of Romano cheese, is readily apparent. Also, the selective liberation of butyric acid by some lipase systems necessitates care in the selection of assay methods. Methods for measuring total lipase activity in these instances should quantitatively measure butyric acid.

Although the differences in the relative proportion of butyric acid in samples which have undergone lipolysis may reflect selective hydrolysis, this term has been qualified by the prefix "apparent." This is necessary since the utilization of butyric acid by other enzyme systems present in the crude preparations could change the relative proportion of butyric acid. In such cases "selective" utilization would be involved. Further work is in progress in this regard and also to determine "apparent" selective hydrolysis of other fatty acids and the factors involved.

TABLE 1

Lipase activity and relative butyric acid content resulting from the action of various lipase preparations on milk fat

	Increase in acid degree	(% butyric acid)		% deviation
		Range	Average[a]	
Pancreatic lipase	16.0	8-9.5	8.7	± 3.0
Freeze-dried raw milk	4.0	12-19	14.7	±15.0
Glandular calf lipase	12.0	32-38	34.5	± 8.3
Glandular kid lipase	18.0	38-45	42.1	± 6.1
Glandular lamb lipase	15.0	42-52	47.6	± 7.5

[a] Average of ten trials.

[1] Technical Note 8-54, Department of Dairy Technology, Ohio Agr. Expt. Station. Supported in part by the Research and Marketing Act of 1946 through the Dairy Products Section, W.U.R.B., A.R.S., USDA, Washington, D. C.

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A SEMI-AUTOMATIC APPARATUS FOR MAINTAINING SOLVENTS WITH CHANGING POLARITY IN CHROMATOGRAPHIC COLUMNS

A number of adaptations have been reported for maintaining a constant flow of a solvent with changing polarity in chromatographic columns *(1, 2, 3, 4)*. During our studies on the identification of organic acids formed during the ripening of cheese it was felt necessary to design an apparatus that would provide a flow of increasing concentration of butanol in chloroform under pressure and at the same time maintain a constant liquid level above the chromatographic column.

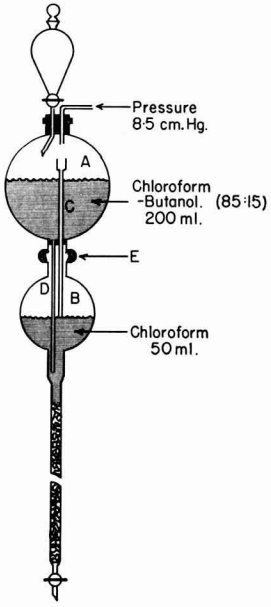

FIG. 1. Diagram of a semi-automatic apparatus for maintaining solvents with changing polarity.

Figure 1 gives the details of such an apparatus. The top reservoir is a 500-ml. flask *(A)* connected to a 100-ml. receptacle *(B)* through a standard ball and socket joint *(E)* by a 1-mm. capillary *(D)* and a 4-mm. tube *(C)*. *C* opens into a funnel in *A* above the 250-ml. level and reaches below the 50-ml. level in *B*. Capillary *D* provides the flow of the solvent from *A* to *B*, which is automatically cut off when the tip of the tube *C* becomes immersed under the solvent. The automatic valve works on the same principle as that described by Hewitt *(3)* for ion-exchange columns but has the additional advantage that it can be worked under pressure.

Table 1 gives the observed and the theoretical proportions of butanol in chloroform obtained during blank fraction collections starting with 50 ml. of chloroform in *B* and 200 ml. of 85:15 chloroform-butanol in *A*. The fractions were collected under a constant pressure 8.5 cm/Hg applied at the top of the column. Density

TABLE 1
Percentages of butanol in chloroform in the eluted fractions

Fraction No.	Observed value	Theoretical value
1	2.20	2.21
2	5.70	5.90
3	8.15	8.20
4	10.20	10.20
5	11.42	11.51
6	12.30	12.47
7	13.05	13.16
8	13.34	13.66
9	13.90	14.03
10	14.00	14.29
11	14.42	14.49
12	14.62	14.62
13	14.75	14.71
14	14.85	14.81
15	14.95	14.85

determinations were made on each successive 16 ml. fractions from 1 to 15. Percentages of butanol in chloroform were calculated from a standard curve obtained by plotting the densities of known mixtures of chloroform and butanol. Averages of three separate runs are reported in the table. The theoretical values were calculated from the formula

$$C_x = \left(1 - e^{-\frac{V_x}{V_o}}\right) C_i,$$

which was derived from the linear equation of the first order

$$\frac{dC_x}{dV_x} + \frac{C_x}{V_o} = \frac{C_i}{V_o}$$

where C_x is the concentration of butanol in chloroform in each fraction collected, V_o the volume of the solvent in *B* (50 ml.), and C_i the concentration of butanol in *A* (15%).

The solvent mixtures obtained in the apparatus as described quantitatively resolve propionic, acetic, formic, lactic, and succinic acids, on a silicic acid column, acidified with 0.5 *N* sulfuric acid (0.7 ml/g).

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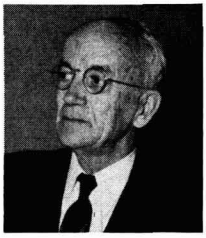
PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

CHARLES WILLIAM HOLDAWAY has given a long period of useful and outstanding service to the dairy industry. He came to Virginia Polytechnic Institute on Jan. 1, 1905. A native of New Zealand, he received his education and training in New Zealand, Australia, Canada, and the United States.

His first assignment at V.P.I. was as manager of the college creamery. The following year he was appointed instructor in dairying and at the same time began taking course work to complete his degree requirements. He received his B.S. from V.P.I. in 1913 and his M.S. in 1915. He was advanced successively to associate professor and professor, and in 1923 was designated head of the newly created Dairy Husbandry Department, which embraced all phases of the work, in both production and manufacturing. He retired as head in 1952 but has continued his interest in the work.



C. W. Holdaway

As a student advisor, Professor Holdaway has been unusually active. The Virginia Tech Dairy Club, later changed to the Virginia Tech Student Chapter of A.D.S.A., was organized under his supervision and with him as advisor in 1921. He was for many years chairman of the Dean of Agriculture's committee for the selection of students for scholarship award and recognition. He is a member of Alpha Zeta, Sigma Xi, Phi Sigma, and Phi Kappa Phi and holds membership in many scientific and other organizations. He is listed in *American Men of Science* and *Who's Who in America.*

Professor Holdaway's work at the Virginia Polytechnic Institute has been outstanding, and he has always maintained high standards in his teaching and research. He was never satisfied to do merely what was required but threw the whole of his strength into the job at hand. He was always ready and willing to accept committee assignments and to assist in every way possible to promote the welfare of the college and increase its service to the people of Virginia.

Professor Holdaway's work on behalf of the dairy industry in Virginia has been a potent factor in the development of the industry in this state. In 1907 at the Jamestown Exposition at Norfolk, Professor Holdaway was one of a small group to take initial action in organizing the Virginia State Dairymen's Assoc. From that day to the present time his wise counsel has influenced the affairs of the association, and he is the only man who has attended every annual meeting of that association through 1954. As a director of that association, he has worked untiringly through the years to aid in solving many vexatious problems.

It is of historic interest to record here the fact that in 1907 Professor Holdaway processed and shipped milk from the V.P.I. dairy plant at Blacksburg to the Jamestown Exposition at Norfolk, 300 miles away. The milk was received in excellent condition. This was an excellent demonstration of the value of proper pasteurization and refrigeration of milk to be shipped long distances.

In 1908 Professor Holdaway tested milk and kept production records and cost accounts on five dairy herds near Blacksburg. This pioneering effort was the forerunner of the Dairy Herd Improvement Association program in the state. In 1915 Professor Holdaway served on an executive committee to complete the organization of the Virginia Creamerymen's Assoc., now known as the Virginia Dairy Products Assoc.

Notable among his research contributions to the dairy industry is his work on the nutrient requirements of dairy cattle, especially the protein requirements of milk cows. Other research work of note includes the feeding value for dairy cattle of many feeds not then commonly used, such as peanut oil meal, soybean oil meal, rubber seed meal, and dried apple pomace; the relative feeding value of various hays; effect of binders on quality of ice cream; net energy of feeds; mineral balances in dairy cattle; and pasture fertilization and management.

Professor Holdaway was among the group that organized the Southern Section of the A.D.S.A. and was its first president. He has continued active in the section work. This group honored him in 1941 for his contributions to dairying in the Southeast. *The Progressive Farmer* chose him as the "Man of the Year in Virginia Agriculture" in 1951. He has been active in community and church work and has for many years been an elder in the Blacksburg Presbyterian Church.

The high esteem in which Professor Holdaway is held in the dairy education field and in the industry is attested to by his many students and friends in all phases of dairy work.

PAUL M. REAVES

Recent Deaths of Members

FRED W. MILNER, specialist in milk and milk products in the Bureau of Dairy Service, California Department of Agriculture (retired), died October 4, at the U. S. Veterans Memorial Hospital at Sawtelle, Calif., after an extended illness.

Mr. Milner, who was born in 1891, graduated from Kansas State College and did graduate work at the University of California at Davis. He was a World War I veteran. He had been assigned to the Los Angeles area since 1928, retiring from the state service in July of this year.

W. N. ANGLE, a successful Guernsey breeder of Rocky Mount, Virginia, passed away August 16. Mr. Angle attended Roanoke College (1927-29). He had been mayor of Rocky Mount, president of the Lions Club, and president of the Virginia Guernsey Breeders Assoc., and was active in 4-H and Boy Scout work.

ARTHUR R. MERRILL, professor emeritus of dairy husbandry, Univ. of Connecticut, died Oct. 5. Professor Merrill, a graduate of the Univ. of New Hampshire, served the Univ. of Connecticut as extension dairyman from 1922-43, and as head of the department 1943-47. He was president of the College Feed Conference Board of the United States and president of the Northeastern section of A.D.S.A. He is survived by his wife, two sons, a daughter, and 6 grandchildren.

GEORGE E. HOLM died suddenly Nov. 11. He retired from the USDA Sept. 1 at the age of 64 after 35 years service. He had served as head of Research Labs. Division of the Bureau of Dairy Industry since 1942. Dr. Holm, an authority on the chemistry of proteins and fats, graduated from Carleton College in 1914. He obtained his M.S. and Ph.D. degrees in biochemistry at the Univ. of Minnesota, completing his graduate work in 1919. He served on the Minnesota staff 1 year before joining the Bureau at Washington. Since his retirement, Dr. Holm has been engaged in the revision of the *Fundamentals of Dairy Science* by L. A. Rogers and associates.

Study of Dairy College Graduates Made by Milk Industry Foundation

The College Relations Committee of the Milk Industry Foundation has released the results of a survey conducted by H. B. HENDERSON of the Univ. of Georgia on "Reaching, Teaching, and Using the Dairy College Graduate." This 28-page report delves deep into the problem of scarcity of students in the field of dairy manufactures and comes up with a mass of data which reveal some of the causes for both lack of students and the reasons why many are unhappy with their chosen field.

The survey also reveals the fact that both industry and colleges are developing plans and programs of recruitment. The inadequacy of staff and laboratory facilities of some schools to give satisfactory instruction in all phases of dairy manufactures also was shown. A loosening of the curricula in most colleges indicates a growing feeling that more opportunity should be given students to become proficient in the field of management.

The answers to questionnaires sent to graduates for the period 1946-53 indicate that, at least during this period of time, graduates in dairy technology are reasonably well paid.

To obtain the graduates' own version of the problem, the group (350) to whom the questionnaire was sent were asked certain specific questions regarding their relationship with the industry. As would be expected, the 294 expressions of opinion differed as to what opportunities the industry offered the college graduate. Often mentioned was the need for an industry training program. Specific recommendations for improving college curricula were given by a large number. A list of interesting comments made by those answering the questionnaire is given in the Appendix.

The report is a good diagnosis of the patient. It is now up to the colleges and industry to correct the things that are responsible for conditions being as they are.

Illinois Drops Product Courses

In an attempt to modernize its training program for students in the field of dairy technology the Dept. of Food Technology at the Univ. of Illinois has changed both its courses and curriculum in this field. The traditional product approach to teaching is being replaced by the more modern method of teaching functions that are common to the manufacture of all products. Realizing that most students in the field of dairy manufactures today are not interested in becoming specialists in any one product of the dairy industry, the Illinois educators believe that more emphasis should be placed on acquainting the student with the basic principles involved in food processing, leaving more time for training in the college of commerce or basic science courses, depending on whether his major interests are in business management or research and technical control.

It is the belief of P. H. TRACY, who is in charge of the dairy technology program at Illinois, that this change in course material and the opening up of the curriculum so as to make it possible for students to better prepare themselves for either management or research work will attract not only more but better students. The disappearance of the small plant, the more complete control of plant labor by the labor unions, and the desire of many of the large dairy corporations to train the young men coming into their organizations in plant operations according to their own requirements have

further emphasized the need for a change in methods of preparing college students for the food industry.

The change in the teaching program at the Univ. of Illinois is based on the results of several years study of the problem in the field and of surveys made among the alumni. It was interesting to find that these former students were almost unanimous in their approval of the proposed changes. Adoption of the functional approach to teaching dairy technology has already been made by the Univ. of California and the Univ. of Florida.

Special Conference on Mineral Metabolism to Be Held

A conference sponsored by the New York Academy of Sciences on calcium and phosphorus metabolism in man and animals, with special reference to pregnancy and lactation, will take place in New York City on Jan. 10-11, 1956. Topics covered will include: calcium balance and turnover studies, parathyroid hormone, problems of parturient paresis in dairy cows, calcium complexing agents, and special aspects of calcium metabolism. F. C. McLEAN, Univ. of Chicago, is general chairman.

Kosikowski Returns from Study in France

F. V. KOSIKOWSKI, Cornell Univ., with his wife and daughter returned in October from a 9-month stay in France as a Fulbright research scholar. While in Europe, Dr. Kosikowski was stationed at the Central Station for Bacteriology and Milk Research, Jouy-en-Josas, near Paris. He collaborated in research with personnel at the station and on new dairy product manufacturing processes. In addition, he traveled through much of France, observing dairy practices and consulting with personnel at the various plants on dairy problems.

Dr. Kosikowski was invited to present a series of lectures at universities in Italy, France, Denmark, Norway, and Sweden. In September he attended the International Dairy Federation meetings in Bonn, Germany. He reports that in Europe there are now available many excellent dairy research laboratories equipped with modern instruments and staffed by outstanding scientists.

Recent Appointments

L. D. BROWN has been named assistant in dairying at the Kentucky Agricultural Experiment Station. Mr. Brown received a Bachelor's degree at Western Kentucky State College and recently received the Master of Science degree at the Univ. of Kentucky. His work will deal primarily with nutrition of dairy cattle.

R. H. BENSON has been appointed extension dairyman at the Univ. of Connecticut. Benson graduated from the Univ. of New Hampshire, received his M.S. from West Virginia, and his Ph.D. from Wisconsin. He previously was engaged in field research with the USDA in Wisconsin. Dr. Benson will be responsible for the extension breeding program and will participate in management, herd health, and housing programs at Connecticut.

J. P. EVERETT, JACK KNEPP, and GERALD BROWN have been appointed research assistants in dairy husbandry at the Univ. of Kentucky. Mr. Everett received his B.S. degree from Alabama Polytechnic Institute and Mr. Knepp and Mr. Brown obtained their B.S. degrees from the Univ. of Tennessee.

The appointment of JOHN WALTON as a research assistant in artificial breeding has been announced by the Kentucky Dairy Department. Mr. Walton is a recent graduate of the Univ. of Kentucky.

E. R. JARMAN has replaced K. T. SCOTT on the Dairy Industry staff at Texas Technological College. Jarman has the B.S. and M.S. degrees from the Univ. of Tennessee and for the past 3 years has been located at the Georgia Mountain Experiment Station, Blairsville, Ga.

Protein Seminar Group Organized

An informal meeting of dairy scientists interested in the chemistry of milk proteins was held Nov. 18-19 at the Allerton House of the Univ. of Illinois. PROFESSOR R. McL. WHITNEY served as chairman of the meeting. Allerton House, located on a country estate near Champaign, is owned by the Univ. of Illinois and is used as a retreat, especially for small group meetings.

Twenty-nine faculty members and graduate students were present for the meeting, which was devoted primarily to informal discussion of research work pertaining to the protein systems of milk. The following universities were represented: Iowa State, Michigan State, Minnesota, Missouri, Ohio State, Wisconsin, and Illinois. The first of these meetings was held in 1954 at the Univ. of Wisconsin. The group voted to meet at Iowa State in 1956 and at the Univ. of Minnesota in 1957.

It was decided to open the seminar meetings in the future to those interested in the chemistry of milk proteins who are located at any of the universities in the geographical area included in the North Central Association of Agricultural Experiment Stations. Invitations will be extended by the host institution prior to each meeting.

Herald Wins Dairy Remembrance Fund Scholarship

C. T. HERALD, graduate student at Michigan State Univ. in the field of dairy manufacture, has been awarded the first research assistance grant of $500 by the Dairy Remembrance

Fund, it has been announced by M. H. LEWIS, president of the fund.

Herald, a graduate of Pennsylvania State Univ., is working toward his doctorate at Michigan State, where his research activities have been concentrated on protein chemistry with special emphasis on the character of the fat globule membrane proteins in milk.

The Dairy Remembrance Fund was incorporated in February, 1954, as a means of honoring men and women in the dairy and allied fields. It is supported by voluntary contributions for that purpose and is dedicated to the advancement of scholarship in the field of dairy education, the support of research and the maintenance of nonprofit institutions in the fields of health, education, and public welfare.

O. E. Reed Honored

OLLIE E. REED, who "retired" this past year after 50 years of service to the dairy industries, nearly half of this time as chief of the Bureau of Dairy Industry of the USDA, was presented with a camera and projector as a token of esteem at the Dairy States Rally and Awards Night ceremonies in St. Louis, Oct. 25. St. Louis was host city to the conventions of Milk Industry Foundation and Intern. Assoc. of Ice Cream Manufacturers the week of October 23-28. The award, presented on behalf of the Dairy Industry Committee by F. BRUCE BALDWIN, vice-president of Abbotts Dairies, Philadelphia, and vice-chairman of the Dairy Industry Committee, was a complete surprise to the recipient.

Mr. Reed's retirement is only partial; he is soon to go to Puerto Rico in a governmental advisory post, and Dr. Baldwin, in presenting the camera and projector, expressed the hope that he would find much enjoyment in taking pictures in years to come, on assignments all over the world.

Arizona Tech. Society Elects Woodward

HAROLD WOODWARD of the Co-Op Dairy, Phoenix, was elected president of the Arizona Dairy Tech. Society for 1956. Others named were JIM RUTHERFORD of the Borden Co. of Phoenix, vice-president; DALE FAULKNER of the Sunset Dairy, Tucson, recording secretary; BOB WEST of the R. L. Perry Co., Phoenix, sergeant-at-arms; and J. W. STULL of the Univ. of Arizona at Tucson, secretary-treasurer. The society meets the second Tuesday of each month, usually in Phoenix.

The Dept. of Dairy Science, Tucson, has completed remodeling the creamery and has made extensive additions to their laboratory facilities. J. W. Stull is acting head of the Department.

Western Section Elects Officers

The ninth joint meeting of the Western Section of the American Society of Animal Production and the Western Division of A.D.S.A. was held at the Univ. of Wyoming, Laramie, July 10-12. Sixty-three papers were given to the two organizations in both general and departmental sessions. Attendance totaled 148 from 18 states, the District of Columbia, and Hawaii. Newly elected officers of the Western Division of A.D.S.A. are: J. O. YOUNG, chairman; L. R. HUNSAKER, vice-chairman; and I. W. SLATER, secretary-treasurer.

The next meeting of the two groups will be held on the campus of the Univ. of Nevada at Reno, July 15-18, 1956.

Washington State Happenings

J. C. KNOTT, who has served as director, Institute of Agricultural Sciences, State College of Washington, since Jan. 1, 1947, returned on July 1 to a full-time program of teaching and research in the Dept. of Dairy Science, in which department he served from 1919 to 1941.

N. S. GOLDING was given the status of emeritus professor of dairy science on Sept. 15. He is, however, continuing research on the development of a subsurface centrifugal hydrometer for nonfat solids of milk. T. L. FORSTER, formerly a staff member of North Dakota Agricultural College, has been appointed to fill the vacancy left by the retirement of Dr. Golding.

I. H. LOUGHARY, dairy specialist, Agriculture Extension Service, has been granted a leave of absence effective Nov. 16. Mr. Loughary will go to Brazil under the sponsorship of the Intern. Cooperation Admin. His project will be to aid in developing a safe milk supply for Racife, Brazil.

MOUNIR NAGMOUSH, who received his Ph.D. degree from the State College of Washington during the past summer, is continuing his studies for another year on a postdoctoral appointment. Dr. Nagmoush is on leave from the Dept. of Dairying, Univ. of Cairo, Egypt.

I. I. LITMAN, a recent recipient of a Ph.D. degree from the State College of Washington, has accepted a postdoctoral fellowship appointment in the Dept. of Dairy Industry, Univ. of California, Davis.

Farmer's Fair Held in Maine

The Ninth Annual Farmer's Fair, held on the Maine campus Nov. 12, attracted a large number of high school students. A milking contest and a fitting and showing contest were sponsored by the dairy cattle class. Various educational exhibits also were featured.

Montana Holds Industry Meeting

The 20th Dairy Industry week under the sponsorship of the Montana Purebred Dairy Cattle Assoc., the Dairy Industry Dept. of

Montana State College, and the Montana Dairy Educational Service Committee, was held Nov. 14-16 at Bozeman. The planning committee was headed by J. A. NELSON, head of the Dept. of Dairy Industry. Speakers included J. C. BOYD of the Univ. of Idaho, E. L. THOMAS of the Univ. of Minnesota, B. R. WEINSTEIN, Crest Foods Co., Ashton, Ill., and M. M. WARNICK, president, American Dairy Assoc., Pleasant Grove, Utah.

Announcements of International Congresses

Eighth Intern. Congress on Animal Production May 23–June 1, 1956, Madrid, Spain. Sponsored by European Assoc. for Animal Production. K. KALLAY, secretary, via Quintino Sella 54, Rome, Italy.

Seventh Intern. Grassland Congress Nov. 6-15, 1956, Massy Agricultural College, Palmerston North, New Zealand. S. H. SAXBY, secretary.

Third Intern. Congress on Animal Reproduction, June 25-30, 1956. Univ. of Cambridge, England. JOSEPH EDWARDS, secretary.

Fourteenth Intern. Dairy Congress, Rome, Italy, Sept. 24-28, 1956. G. PITTONI, secretary general, via Guidubaldo del Monte, 24 Rome, Italy.

International 4-H Dairy Cattle Judging Contest Won by Michigan

Paced by JAMES DEAN, the Michigan 4-H Dairy Cattle Judging Contest, held in conjunction with the Intern. Dairy Show in Chicago, took high honors. Dean was high individual. Second team was New York, and the Indiana team placed third. The coach of the Michigan team was CLAYTON INGERSON, assistant county agent, Adrian.

Completed Theses

M.S. Degree:

JACK L. ALBRIGHT—The influence of calcium borogluconate on the blood levels and urinary excretions of calcium in dairy cows with parturient paresis. Washington State College.

LEONARD D. BROWN—Effect of protein level of calf starters on the growth rates and metabolism of young dairy calves. Univ. of Kentucky.

GEORGE F. FRIES—The value of corn cobs and cottonseed hulls on roughages for dairy heifers. Univ. of Kentucky.

PAUL A. PUTNAM—The nutritive value of certain fibrous feeds for dairy cattle. Washington State College.

Ph.D. Degree:

DIPTIMAN CHAKRAVARTI—The protein stability and wettability of milk powder as affected by changes in milk composition. Washington State College.

WILBURN E. GLENN—Bacteriological studies of cultured buttermilk as associated with pH levels and acetylmethyl-carbinal plus bracetyl content. Washington State College.

IRVING I. LITMAN—A study of a fat-protein complex in powdered milk. Washington State College.

MOUNIR R. NAGMOUSH—The nitrogen source and the oxidation reduction potential as they apply to mold growth. Washington State College.

BALBIR KRISHMA SONI—A study of the excretion of certain steroid hormones at the time of parturition in normal cows and in cows affected with parturient paresis. Washington State College.

OUR ASSOCIATION

Enough Responsibility for All

Dear Fellow Members:

One of our officers wrote recently, "Association work is sometimes most discouraging. I seem to be 'a voice calling in the wilderness' with so few of the members showing real enthusiasm and giving definite assistance in connection with the Association's programs."

This is a natural reaction of those who work with groups of people. It is the story told countless times by association secretaries as a result of their experiences with membership activities. It is the complaint of committee chairmen as they attempt to engender enthusiasm and activity on the part of their committee members. As one veteran trade association secretary said, "I learned from the early frustrations in my career that if I were to continue in association work I had to adopt the philosophy that only a small percentage of the members may be depended on to carry the load."

Is this failure of association members to respond an indication of apathy or inertia—or is it because of the individual's lack of understanding or opportunity? Perhaps each of these is involved, at one time or another and in different degrees.

It may not be illogical to assume that there are both ambition and inertia in each of us—and that whether we stay at rest or continue in forward motion depends upon the balance of the two. The disproportionate quantity of the "static" quality in the majority of us may be the principal reason for the statement that "only one of every 10,000 persons demonstrates leadership (or executive) ability."

Is it because of apathy and inertia that the final goal of many persons appears to be to *belong* to an organization, or to *become a member* of a committee, or to *accept* an officership, rather than the subsequent contributions which may be made in these respective capacities? Surely, we are all guilty of adopting, with little or no resistance, the philosophy of "Let George do it" and are content to become a rubber stamp for whatever George does.

On the other hand, there are members in our Association whose full potential to serve has never been realized, either because they have been inadequately informed or because they have never been invited to participate in a specific way.

All of the foregoing merely points up a major responsibility of your officers—the responsibility to create more enthusiasm and interest among all of our members in the affairs of the Association, to seek out those with the ability and the desire to serve the Association and to present opportunities to them, and, finally, to motivate those who are now in important positions. Webster defines inertia as "the property of matter by which it will remain at rest . . . unless acted upon by some external force." It is the officers' responsibility in our Association, as in others, to supply the basic elements of this force, if not the force itself.

At no period in the history of our Association is the time more propitious for us to shake off whatever apathy we may have and assert ourselves more positively among the influential groups of the industry. Over the years, in keeping with the progress of the industry, our Association has increased in size and in stature—but these changes are small in comparison to those which are immediately ahead. Scientific and technological advances in the next 50 years will be greater than we can now envision, and demands on science will be increasing at a tremendous rate. Therefore, our Association, as the educational and scientific organization of the industry, may logically assume a more important leadership role than it has ever before experienced—if it is prepared to do so.

An immediate need is for us to give increased attention to the membership and subscription campaign now in progress. Some of the regional and state programs are already reaping the harvest, others are much less successful. There are many qualified persons known to each of us who are ready to join, awaiting only a personal invitation. There are dozens of dairy companies and organizations in our own area which are willing to subscribe to the Journal if they are advised of its value by someone known to them personally. Each of us then may ask this question, "What have I done this year to get a new member or subscriber?"

In addition, those of us in education should initiate definite action within our individual departments to increase greatly the student affiliate membership. There is something wrong in our teaching methods if our students at the sophomore level and above are not using the Journal for classroom assignments. How else are they to be kept current in respect to research and research leaders in their major field? Therefore, is it not to be expected that many of these students (especially the upperclassmen) will affiliate themselves now with their own professional organization if they are invited to do so? The least we can do, as student advisers, is to encourage our students to accept early their responsibilities and privileges and to become familiar with the organization which, some day, will be in their charge. Here is a great challenge for us in education who are responsible for pointing out the pathway these young people should take to professional leadership.

The greatest single enterprise of our Association is the Journal, which utilizes approximately 80% of the annual budget. However, the vast majority of us tend to take the Journal for granted—and we are unaware of the constant pressure which exists to meet the monthly deadline, to have in each issue the maximum of valuable copy effectively presented, and to ade-

quately finance the publication. It is in respect to the cost that each of us should give close attention. Dues and subscriptions do not supply sufficient funds, and advertising is necessary in ever increasing quantities if the steady improvement in the Journal is to continue. As individual members we can help with the advertising by personal contact with those who should be using advertising space in our publication but, even more, by reading the advertising in the Journal and supporting these advertisers on every occasion. In this connection, can we not each devote ten minutes per issue to this advertising activity?

The Journal is in the process of some "face lifting," which will make it more attractive and marketable. This will cost money. Also, the special commemorative issue for June, containing some 300 pages, is an expensive venture that will require heavy advertising support if it is to be self-financing. We should not allow the Journal Management Committee and the Editor to be burdened with full responsibility for these financial needs.

Another privilege that we have, as members, is to submit our views to Editor Tracy for inclusion in the "Letters to the Editor" section of the Journal. Here is an opportunity for free expression which, if utilized, will result in a stronger and more useful Association.

The great desire of your officers is to have each member experience the friendly atmosphere which has been greatly responsible for the success of our Association. One major way of accomplishing this is for the member and his family to attend the annual meetings. Recently, one of our members, an industry public relations leader, said, "I believe the best way to get to know the Association and appreciate its worth is by attending the annual meetings. My family and I have attended regularly for many years and I wouldn't miss it. It is always a great inspiration to me."

And for you who have attended the annual meetings, what are your suggestions for making the general sessions and the technical programs more effective? The Program Committee, chairmanned by Ralph Erb of Washington, is planning now the details for the Connecticut meeting and would welcome your views. How would you suggest that the contributed technical papers be selected and presented? What symposia do you think should be included? What should the special session on "Dairy Education" contain? When should the awards program be held—the second or last night? What features should be planned in commemoration of the GOLDEN JUBILEE meeting?

One of the Association's special committees this year with O. F. Garrett as chairman is concerned with reviewing and standardizing the procedures used for the major Association awards. The results of this activity will constitute a definite step forward in the administration of this phase of our Association's affairs.

Cordially yours,

I. A. GOULD

STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

Thomas to Edit Student Affiliate Section

E. L. Thomas

Beginning with the January issue of the Journal, E. L. THOMAS, associate professor of dairy industry at the Univ. of Minnesota, will edit the Student Affiliate Section, which is to be a regular feature of the Journal. All news intended for this new department should be sent directly to Professor Thomas.

Nine Dairy Products Teams Participated in International Dairy Show Contest

The Iowa State College team was first in the Intern. Dairy Products contest held at Chicago in conjunction with the Intern. Dairy Show. The contest was sponsored by the Chicago Dairy Technology Society. Ohio placed second, and South Dakota was third. JIM RIEKENS, Iowa State College, was high individual with a score of 13; RON PERKINS, Ohio State, was second (22), and CHARLES SAPP, South Dakota, was third (28).

The winning teams and individuals for each of the products were as follows:

Product	*High Team*	*High Individual*
Milk	Iowa State	TOM RUZIKA (Iowa State)
Butter	South Dakota	CHARLES SAPP (S. Dakota)
Cheese	Ohio State	JIM RIEKENS (Iowa State)
Ice Cream	Iowa State	DICK MARQUARDT (Wisconsin)

Illinois Places First at International Dairy Cattle Judging Contest

For the second consecutive year the Univ. of Illinois Dairy Cattle Team, coached by E. E. ORMISTON, placed first at the Intern. Dairy Cattle Judging Contest with a score of 2,081. Kansas State College was second (2,070) and the Univ. of Wisconsin was third (2,052). The three high individuals were PAUL BRANDL of Wisconsin, JOE BICKNELL of Illinois, and KENNETH KIRTON of Kansas State College.

The first place teams and individuals for each of the breeds were as follows:

Breed	*High Team*	*High Individual*
Ayrshire	Kansas	JOE BICKNELL (Illinois)
Brown Swiss	Ohio State	BELVEDERE LOVELY (Ohio State)
Guernsey	Wisconsin	PAUL BRANDL (Wisconsin)
Holsteins	Purdue	DON PROFFITT (Missouri)
Jerseys	Illinois	JOHN HOSTETLER (Michigan State)
Milking Shorthorn	Iowa State	ANCEL ARMSTRONG (Kansas)

Sixteen teams participated in the contest which was held in conjunction with the International Dairy Show at Chicago.

Collegiate Students' International Contest Held in St. Louis

With 26 teams from land grant colleges in the United States competing in the annual contest sponsored by DISA and A.D.S.A. held at St. Louis Oct. 25, top honors again went to Mississippi State College. This win gives the southern team, coached by F. H. HERZER, permanent possession of the coveted "All Products Bowl," which they had won on two previous occasions. It also means that one of the 3 team members will be entitled to a $1,380 scholarship.

Other placings in the contest were:

Second—Iowa State College, coached by W. S. ROSENBERGER, which received a fellowship worth $1,280 and the Cheese Cup;

Third—South Dakota State College, coached by R. J. Baker, which received a $1,180 fellowship and the Ice Cream Cup;

Fourth—Ohio State Univ., coached by W. L. SLATTER, which received a $900 fellowship.

The team cups for milk and butter judging went to two teams which did not place in the "top four" for all around performance in the contest. The Milk Cup went to the team from the Univ. of Connecticut, whose coach, L. R. DOWD, is himself a former contestant. The Butter Cup was taken by the Univ. of Tennessee, coached by H. N. CARRINGER.

Awards to the leading teams were presented at Awards Night, Oct. 26. The setting for the affair, which honored both the young college students and the state dairy associations which represent the industry regionally, was a "political convention." Cheers by the state association representatives for their "favorite sons"—the college students—sparked an evening of songs, surprises, and awards, arranged as a joint entertainment event by Milk Industry Foundation, Intern. Assoc. of Ice Cream Manufacturers, and Dairy Industries Supply Assoc.

On the Mississippi State team were two of the top rated individuals in the contest. JOE A. SIMS, of Starkville, Miss., was proclaimed the best all-round judge of dairy products in the world, and was presented with a Gold All Products Medal. L. B. BARTON, of State College, Miss., who was originally scheduled only as an "alternate" member of the Mississippi team,

was entered in the contest at the last moment, and acquitted himself so well in butter judging that he received the gold medal in this category.

The second-place team—Iowa State College—which has always been a leading contender for prizes in the unique contest, also boasted two students who took medals for individual judging performances. They are JOHN POLLEI, a 21-year-old native of Fairmont, Minn., who was awarded bronze medals for cheese and butter judging; and JAMES RIEKENS, a 20-year-old native of Belmond, Iowa, who received the bronze medal for ice cream judging.

The third place team—South Dakota State College—also had two individual medalists on its roster. They were CHARLES W. SAPP, a 21-year-old senior of Brookings, S. D., who received the bronze medal for judging all products; and KENNETH C. SEAS, a 25-year-old navy veteran of White, S. D., who took the silver medal for ice cream judging.

On the fourth place team—Ohio State Univ. —was RONALD PERKINS, a 21-year-old senior of Springfield, Ohio, who received the silver medal for all products judging and the gold medal for ice cream judging.

Other individual medalists in the contest were:

From the Univ. of Georgia team, WALTER E. VERITY, JR., a 25-year-old Air Force veteran from Long Island, N. Y., who received the gold medal for cheese judging.

From the Univ. of Maryland, CALVIN S. TABLER, a 24-year-old senior from Dunellen, N. J., who received the bronze medal for milk judging.

From the Univ. of Minnesota, EDWARD E. BRUGLER, a 20-year-old junior from St. Paul, Minn., who received the silver medal for butter judging.

From Cornell Univ., Ithaca, N. Y., DONALD S. GAY, a 21-year-old senior from Owego, N. Y., who received the silver medal for cheese judging.

From North Carolina State College, CHARLES T. WEATHERLY, a 22-year-old senior from Raleigh, N. C., who received the silver medal for milk judging.

From Pennsylvania State Univ., RICHARD MONG, a 20-year-old junior from Seneca, Pa., who received the gold medal for milk judging.

C. J. BABCOCK, of the Foreign Agricultural Service of the USDA, served as the superintendent of the contest. D. R. STROEBEL, also of FAS-USDA, served as assistant superintendent. Official judges were: Milk—D. A. PETTEE, The Creamery Package Mfg. Company; Butter—N. E. FABRICIUS, Ladysmith (Wisconsin) Milk Producers Cooperative Association; Cheese—H. L. WILSON, Kraft Foods Co.; Ice Cream—J. HOFFMAN ERB, The Borden Co.

Chairman of the Committee representing A.D.S.A. in arrangements for the contest was G. M. TROUT of Michigan State University. CHARLES WEINREICH, Cherry-Burrell Corp., headed a similar committee in DISA.

Jakeman Named Honorary Dairy Club Member by Massachusetts Students

At the second annual Dairy Club Breakfast held Oct. 29 at the Univ. of Massachusetts, BROOKS F. JAKEMAN was named Honorary Dairy Club Member. Mr. Jakeman is Northeast District Manager for the Cherry-Burrell Corp. and has just completed 35 years of service with that firm. The Dairy Club cited Mr. Jakeman for "his deep interest in developing ability in young people, his many years of experience in the dairy industry, and his loyalty to his Alma Mater."

Mr. Jakeman was graduated from the Univ. of Massachusetts in 1920. He excelled in football, basketball, and baseball. In the same year of graduation he started working for the Wright-Ziegler Co., which later became affiliated with Cherry-Burrell. His 35 years of service have been continuous. In this period of time he has been a part of many new developments in the industry, including the continuous ice cream freezer, the Vacreator, and the high-temperature short-time pasteurizer.

Dairy Club co-presidents, C. W. JOHNSON of Southboro, Mass., and J. J. DONOVAN of Boston, Mass., acted as masters of ceremony.

Michigan State Alumni Meet

The 14th annual Homecoming Breakfast of the MSU Dairy Club was held on October 22. One hundred twenty dairy alumni and friends gathered at the Kellogg Center for breakfast and then were given a preview tour of Anthony Hall, the animal industries facility now being constructed.

Activities of State College of Washington Students

Dairy cattle judging teams from the State College of Washington competed at the Pacific International, Portland, Ore.; at the Dairy Cattle Congress, Waterloo; at the Intern. Dairy Show, Chicago, and at the Grand National Livestock Exposition at San Francisco. The expenses involved in making these trips were partially met by the sale of heifers fitted by members of the Student Branch of A.D.S.A.

Dairy Products judging teams competed at the Pacific International and the Intercollegiate Dairy Products Judging Contest at St. Louis. Trip expenses, in part, were met by the sale of ice cream bars and bottled milk in vending machines installed in a college dormitory.

ROBERT H. KOSOLA, a senior in dairy technology, has received the Agricultural Leadership Award for Region 5 given by the Milk Industry Foundation. Mr. Kosola is a veteran of 2 years service in the Armed Forces.

Kentucky Faculty Entertain Students

Over 80 students and faculty members attended the "bean" feed at the annual student-faculty mixer arranged by the Dairy Section of the Univ. of Kentucky. All students interested in dairying were invited. Included on the menu were steak sandwiches, scalloped potatoes, cottage cheese, pickles, olives, ice cream, cake, and all the milk the boys could drink.

T. R. FREEMAN headed the committee on arrangements and served as toastmaster. He welcomed the returning Dairy Cattle Judging Team by informing the gathering that the team had placed sixth in the contest at the National Dairy Cattle Congress at Waterloo, Iowa.

E. C. SCHEIDENHELM, field agent in dairying, entertained the group with a demonstration of how to place a class of "bulls." LEE DEAN, high school freshman from Harrodsburg, presented a ventriloquist act superior in technique to that of many adult artists.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

The Relation of Adequate Nutrition to Atherosclerosis[1]

F. A. KUMMEROW

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The need for adequate amounts of carbohydrates, fats, proteins, minerals, and vitamins for optimum nutrition has been amply demonstrated. However, knowledge of the interrelationships and the possible roles of these nutrients in metabolic disorders is still fragmentary. Some investigators believe that one of the most prevalent metabolic disorders, atherosclerosis, may have its origin in an excessive dietary intake of fat and cholesterol. On the other hand, faulty carbohydrate metabolism or a deficiency in certain amino acids or vitamins has also been implicated in this disease. For example, diabetics are more inclined to have atherosclerosis than non-diabetics, and rats and monkeys which have been fed a methionine or pyridoxine deficient diet have been reported to show signs of atherosclerosis.

Human atherosclerosis, which is the most prevalent type of arteriosclerosis, is responsible for 25 per cent of all deaths in the United States. Arteriosclerosis is a condition that is marked by a loss of elasticity, thickening, and hardening of the arteries. The term atherosclerosis refers to deposits of lipoidal and plaque-like materials in the arteries. A chemical analysis of this lipid material has shown that it is composed of 27 per cent cholesterol, 42 per cent cholesterol esters, 16 per cent phospholipid, 10 per cent neutral fat, and 4 per cent galactosides. Since 1908 it has been known that deposits of lipid material are found in arteries of herbivorous animals (such as rabbits) which have been fed large amounts of cholesterol. Similar observations have been noted in chickens and dogs; in all of these animals, the serum cholesterol level exceeded 500 mg. per cent. Therefore, it seemed logical to rely on the serum cholesterol level as an index of faulty cholesterol metabolism and a sign of possible atherosclerosis.

Cholesterol and lipoprotein determinations of human blood serum seem to indicate a correlation between fat intake and atherosclerosis. Clinical observations have shown that a high correlation exists between obesity and atherosclerosis. Attempts to establish a relationship between cholesterol intake and human atherosclerosis may thus seem logical. However, this assumption is over-simplified and has been questioned by many investigators, because cholesterol can be readily synthesized from fat, carbohydrate, and certain amino acids *in vivo*. Although the liver is the major site of cholesterol synthesis, other tissues, including arterial tissue, are also capable of this process.

Cholesterol Level

An excessive caloric intake may therefore be more directly related to cholesterol synthesis than a high-fat, high-cholesterol diet. The synthesis of either fat or cholesterol would not be restricted by consuming a low-fat, low-cholesterol diet unless the caloric intake is drastically curtailed. A low-fat, low-protein diet such as the rice-fruit diet, if fed at a level below the recommended minimum daily caloric requirement, leads to a lower blood cholesterol level but it also leads to a negative nitrogen balance. This diet is not well enough tolerated to be used continuously.

A fairly constant level of cholesterol is maintained in each individual under normal conditions. The level can normally be as low as 110 or as high as 310 mg. per cent. If an excessive amount of cholesterol is consumed, it is quickly removed from the blood. When the level of intake is inadequate, cholesterol is synthesized. The actual precipitation of cholesterol and cholesterol esters on the arterial wall may be governed by the law of mass action and may serve as a last desperate means of maintaining a normal blood cholesterol level. When precipitation occurs in a large artery such as the aorta, it will take many years before serious damage occurs. However, when cholesterol precipitates in the small coronary arteries, blood flow through the myocardium or heart muscle is impeded and serious damage can occur in a relatively short period of time.

Cholesterol is present in the blood as esters of fatty acids, in combination with phospholipid

[1] Reprinted from *Food and Nutrition News*, Vol. 27, No. 1. October, 1955.

and protein or dissolved in neutral fat as a component of the chylomicrons. The intrinsic combinations of these various factors are still not well understood but a few facts are known about them. It is known that the kinds of lipoproteins in the blood have an important bearing on the synthesis of the cholesterol lipoprotein complex. Furthermore, phospholipid seems to aid in emulsifying cholesterol and thus may help to prevent precipitation of cholesterol on the arterial wall.

Type of Fat

The degree of saturation of the dietary fat also seems to influence the blood cholesterol level. Chickens fed 0.5 per cent cholesterol and 10 per cent saturated fat (hydrogenated cottonseed oil i.v. 93) had a higher blood cholesterol level than those fed highly unsaturated cottonseed oil (599 and 303 mg. per cent respectively). See Table 1. Furthermore, chickens which had been fed 0.5 per cent cholesterol and 10 per cent hydrogenated vegetable fat had as high a cholesterol level as those fed 0.5 per cent cholesterol and animal fats such as lard or butter fat. Similar results have been noted in rabbits and in man. It is therefore incorrect to state that animal fats are more atherosclerotic than vegetable fats, as some investigators have interpreted these data.

These results also indicate that an adequate supply of dietary fat containing highly unsaturated fatty acids may actually help to prevent an elevation in blood cholesterol level. Animals and man are incapable of synthesizing the highly unsaturated "essential" linoleic and arachidonic acids from saturated fatty acids, carbohydrates or proteins. Dietary sources of these essential fatty acids may therefore be necessary for the formation of the highly unsaturated cholesterol esters in normal blood.

TABLE 1

The degree of unsaturation of the dietary fat and the blood cholesterol levels in chickens

Fat	Iodine values	Blood cholesterol
		(mg. %)
No additional fat	—	261
Cottonseed oil	115	303
Hydrogenated cottonseed oil	93	599
Lard	62	546
Butter fat	33	541

Adequate Diet

In conclusion, it is apparent that either an inadequate diet or an unbalanced hormone therapy has always been necessary to induce experimental atherosclerosis. Therefore, it can not be overemphasized that a balanced diet adequate in fats, protein, minerals, and vitamins may serve as the best insurance against atherosclerosis. Such a diet will contain cholesterol and animal fats because eggs, milk, and meat products serve as the best sources of these essential nutrients. If consumed in moderation and with vegetables as a source of plant sterols to partially complex or tie up the dietary cholesterol, such a diet would be more apt to insure maximum lipoprotein, phospholipid, and cholesterol ester synthesis than one deficient in protein and high in carbohydrate. The latter type of diet may lower blood cholesterol levels but does not give any assurance that the excess cholesterol has been metabolized and not added to the existing deposits already on the arterial wall.

New Developments in Bactericides

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Bactericides of greatest interest in the food and institutional sanitation fields at the present time include the hypochlorites, quaternary ammonium compounds, chloramines, dichlorodimethylhydantoin, and the iodophors. The research reported on these products in recent years is too extensive to cover at this time. Therefore, the present discussion will deal only with certain phases that may serve to illustrate the trend toward new or improved products and their specific application in sanitation fields.

An interesting characteristic of all of the above compounds is their dependency on one of the three halogens, chlorine, bromine, or iodine. When the halogens are employed alone for bactericidal purposes in solutions free of organic matter, chlorine appears to be the most active, with bromine next and iodine slowest. However, in the presence of organic matter, iodine usually is more active than chlorine. Iodine also can be combined with certain surface active agents to form an iodophor, a new type of product which has occasioned considerable interest in recent years as a germicide. Dichlorodimethylhydantoin represents an effort to provide an active form of chlorine in combination with a complex organic compound. The result is a germicide which approaches the hypochlorites in activity. In quaternary ammonium compounds the complexity of the molecule provides additional opportunities for changes in chemical configuration that may result in an improved germicide. Research is continually turning out new germicidal products, some of

which appear to offer promise of faster bactericidal action. Examples of new products are chloromelamine and trichlorocyanuric acid.

Factors Affecting Activity of Bactericides

Most of the factors affecting activity of bactericides have been studied over a period of many years. They include effect of type of organism, time of action of bactericide, concentration and temperature of the bactericidal solution, and effect of inhibitors like organic matter, hard water salts, and anionic surface active agents. Sufficient information is available on such factors so that no further discussion of the general subject will be attempted here. Some factors, however, have received less attention, especially with regard to some of the newer types of bactericides, and perhaps should be discussed briefly from standpoint of their effect on application of specific products for certain bactericidal procedures.

Potentiation of bactericidal action. The possibility of accelerating activity above the normal by adding other chemicals to a bactericidal solution has always been an intriguing field for speculation. One such attempt has been the addition of wetting agents to hypochlorites. However, laboratory studies in which various wetting agents were added to representative hypochlorite solutions indicated no accelerating effect on either nonsporeforming bacteria or bacterial spores. This does not preclude the possibility that under some conditions application of the hypochlorite may be rendered more effective through better wetting or spreading properties when a wetting agent is incorporated in the hypochlorite solution. This is purely a physical effect. Considerable care should be used in selection of the wetting agent for such purpose, however, as some rapidly neutralize hypochlorites and nullify their bactericidal activity. Some mixtures of hypochlorite and wetting agent apparently need to be adjusted to higher than normal pH levels to stabilize the hypochlorite in the presence of the wetting agent. This has resulted in a decrease in bactericidal action through the higher pH of the hypochlorite solution.

An interesting example of bactericidal potentiation is the effect on quaternary action of complex phosphates, like tetrasodium pyrophosphate and tripolyphosphate, and chelating agents, such as Versene. Addition of such agents to quaternary solutions markedly accelerates bactericidal activity through some mechanism not yet definitely established. Such potentiation occurs both in distilled water solutions and in preparations of the bactericide made up in waters containing hard water salts. This addition can be employed to at least partially offset the inhibiting effect of hard water salts on quaternary action. Electron micrographs of cells treated with such sequestering and chelating agents suggest the possibility that they may alter the cell surface in such a way that the quaternary can more easily penetrate the cell.

Effect of pH on bactericidal action. No explanation is available for the varied response of different species of bacteria to pH of quaternary solutions. In general, species like *Pseudomonas aeruginosa* are more susceptible to quaternaries at low pH levels, and *Micrococcus pyogenes* var. *aureus* and *Escherichia coli* are more susceptible at high levels. Such information does enable adjustment of quaternary solutions for greater activity in specific applications where a particular type of organism, as for example *Ps. aerguinosa* or *Ps. fluorescens,* may present a problem in destruction.

Recent improvement of some commercial hypochlorite preparations has been accomplished through reduction of excess alkalinity and consequent stabilization of use dilutions at lower pH levels. The result is greatly enhanced bactericidal activity in use dilution based on more rapid release of the active agent, hypochlorous acid. For example, a hypochlorite standardized to provide a pH of 8.0 at 100 p.p.m. may be many times as active as one at pH 9.0 or 10.0.

Effect of pH on iodophor activity against bacteria is also of interest at this time. Results indicate that bactericidal activity of the iodophors greatly decreases with an increase in pH. Solutions at pH 3.0 and 5.0 show decidedly greater activity than at pH 7.0. The iodophor therefore must be used at pH 5.0 or lower for maximum bactericidal activity and is relatively slow at pH 7.0 and above. This fact is of special significance in considering effect of alkalinity of the water on pH of use dilutions of low concentration (such as 12.5 to 25 p.p.m.) iodophor solutions. Some waters may raise the pH of a 12.5 p.p.m. iodophor solution above 7.0 unless the product is highly buffered at a low pH.

Mechanism of Action of Bactericides

Studies have shown iodophors to exhibit a high rate of bactericidal activity against nonsporeforming bacteria. When different products are compared against organisms such as *M. caseolyticus, Ps. aeruginosa, E. coli,* and *S. lactis,* the iodophors at pH 5.0 generally will show a higher rate of destruction than hypochlorites at pH 8.0 and the hypochlorites show greater activity than quaternaries at the same pH level. However, when bacterial spores are employed as the test agent, a different order of activity may occur. Preliminary studies indicate that most rapid spore destruction occurs with hypochlorites, with a relatively poor rate of destruction by iodophors and quaternary compounds. A somewhat similar relationship appears to exist in lactic bacteriophage. Hypochlorites exhibit a high rate of destruction of phage in laboratory trials. Quaternaries appear next in order of activity on a p.p.m. basis, and iodophors seem to be the slowest of the three types of compounds in rate of destruction of phage.

The comparative rates of destruction of nonsporeforming types, bacterial spores, and lactic bacteriophage by the three bactericides discussed above do have considerable practical significance, especially in dairy and food processing plants, where bacterial spores or bacteriophage may represent serious problems. It also suggests some interesting contrasts in mechanism of action of the different compounds and possibly, in the case of spores, a relationship between size of molecule and rate of penetration of the compound. It is generally believed that hypochlorites cause an irreversible oxidation of one or more of the cell enzymes needed for respiration. Some workers also believe death of the cell may be caused by a general chemical reaction between chlorine and bacterial protoplasm. Results on spores and bacteriophage suggest that either mode of action of the iodine products differs from that of chlorine or that the iodine molecule is less able to penetrate to the vital substance of spores or phage in order to destroy them. It is known that hypoiodous acid is much less active than hypochlorous acid and molecular iodine, but, in hypochlorites, the primary active agent in destruction of nonsporeforming organisms is believed to be hypochlorous acid.

Less is known about the mechanism of action of quaternaries, although more studies have been reported on them than on the hypochlorites. One explanation for their action is that the quaternary disorganizes the cell membrane and denatures certain proteins essential to metabolism and growth. It also has been suggested that quaternaries may inactivate specific enzyme systems essential for respiration of the cell. Still another explanation is that the high rate of adsorption of the quaternary on the cell surface disrupts cell processes by causing a leakage of cellular constituents through the cell wall. There is some evidence that high resistance of some nonsporeforming types of bacteria to quaternaries is related to resistance of the cell membrane to penetration of the compound to vital portions of the cell.

Equipment for Field Handling Frozen Semen

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Many workers have demonstrated that frozen semen can be used satisfactorily in artificial insemination of dairy cattle and that it has many advantages. However, some artificial breeding associations have been reluctant to use frozen semen because of the large consumption and high cost of solid CO_2 to the technicians and the unsatisfactory field handling equipment.

Satisfactory subzero cabinets should conform to the following principles:

1. Maintain an even, low temperature.
2. Use a minimum of solid CO_2.
3. Be able to store a maximum number of ampules in the space available.
4. Have a large CO_2 capacity.
5. Ampules should be easily accessible and easy to identify.

The equipment described hereafter was designed along these principles and fits into the following sequence of handling frozen semen (all semen prior to freezing is handled in a coldroom 5° C.).

1. Semen is extended and glycerolated.
2. Extended semen is hermetically sealed in glass ampules.
3. Ampules are placed in holders or cartridges.
4. Cartridges and ampules are placed in a circulating alcohol bath and frozen.
5. Holders and ampules are stored in a large subzero cabinet. They are placed in an alcohol bath in which there is a continuous supply of solid CO_2. This maintains the alcohol at a constant temperature several degrees Centigrade lower than the air temperature of the cabinet. This method makes it possible to maintain this low temperature even if the amount of solid CO_2 becomes quite small. The ampules will remain at the same temperature at all times.
6. Holders and ampules are transferred to the technician's storage cabinet.
7. Technician transfers the holder and ampules to a gallon Thermos jar for field use.

Description of Equipment

The technician's subzero cabinet is 29 in. wide, 43 in. long, and 34 in. high, plus the lid, which is 6 in. high. As Santocell A is highly hydroscopic, it is necessary to take precautions against vapor entering the insulating material.

The cabinet is constructed from ⅜-in. marine plywood, covered on the inside with ¼-in. (Figure 1) roofing asphalt and two layers of vaporproof refrigerator paper. In order to prevent vapor transfer, all wooden joints of the box proper are put together with Weldwood glue, with the exception of the box top, which is fastened with brass screws and sealed with #800 Marine Sealer, and the outside is covered with galvanized iron and all joints are soldered. The iron is bent over the top of the box 4 in. from

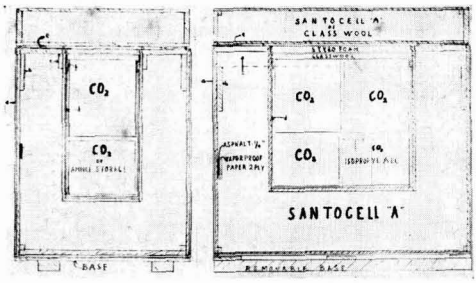

FIG. 1. Cross section of subzero cabinets.

the outer edge, a layer of #800 Marine Sealer (manufactured by Minnesota Mining & Smelting Co.) is placed under the edge, and then the iron is fastened into place with wood screws. This leaves about 4 in. of wood exposed and breaks the heat conduction of the iron.

The inner box is also lined with galvanized iron, and all seams are soldered. A ½-in. by 2-in. strip of wood is bolted 2 in. down from the top (Figure 1) to support the cans that hold the solid CO_2. These cans are 11 in. by 11 in. by 12 in. deep (Figure 2) and hold one entire solid block of CO_2. If necessary, the CO_2 can be crushed. A 1-in. layer of Styrofoam is fastened to the inside of the lid. A 1-in. glass wool mat is placed over the cans of CO_2. Partitions are placed in the alcohol-tight storage can (Figure 3) to hold ampule cartridge, A. The basket, B, which holds the CO_2 is shown in place.

All processing is done in a 5° C. coldroom. The ampule holders or cartridges are easily loaded. Each ampule is slipped individually through the slot in the holder. When the ampule is turned 90° it rests snugly in the holder, touching only the ampules on either side. Each holder contains 17 ampules. The holders fit tightly together with a minimum waste of space. One hundred eighty-seven ampules fit into 11 cartridges and take up a space 8½ in. long by 2⅛ in. wide and about 6 in. deep. A specially designed clip on the partially filled cartridge holds the ampules in place during the freezing process. Individual identification of each ampule and cartridge is possible.

Each cartridge is 8½ in. long and fits into a standard gallon-size Thermos jar for field use. Since this length is not economical for large central storage facilities, the cartridges are so constructed that they may be hooked together. The double units are then placed on a rack, and the entire assembly fits in the circulating alcohol freezing bath. A continuous freezing curve

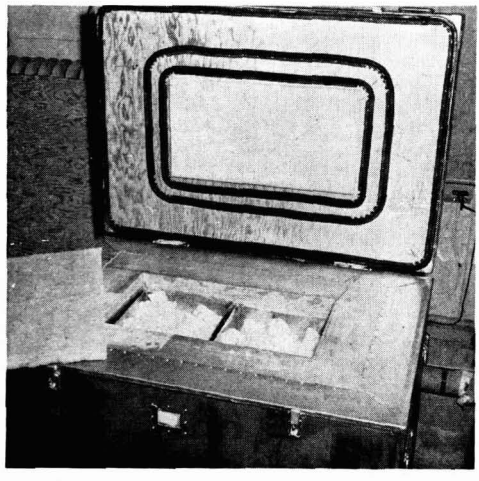

FIG. 2. CO_2 in place in subzero cabinet.

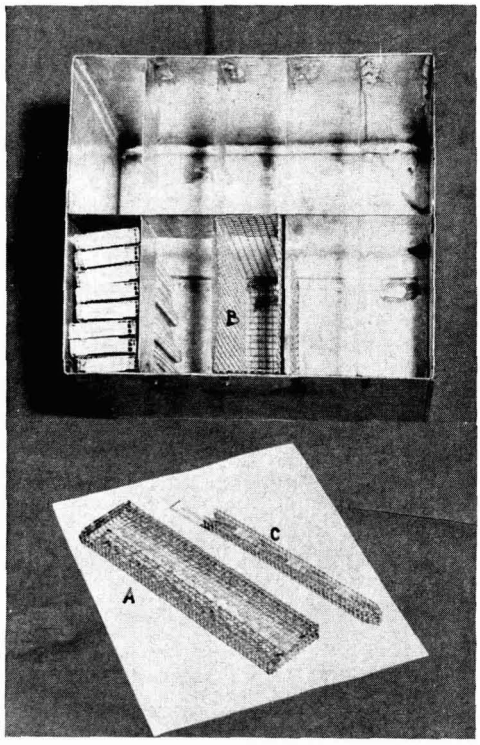

FIG. 3. Ampule cartridges and storage can arrangement.

is recorded by a Leeds & Northrup Speedomax recorder for each batch of semen frozen.

A standard gallon Thermos jar is used in the field by technicians. The large cartridge, A, as shown in Figure 3 is used when large amounts of semen from a single bull are to be stored. The smaller cartridge, C, is loaded from the larger units. This can be done without removing either cartridge from the subzero cabinet by using two pairs of long handled forceps, thus preventing changes in temperatures of the ampules.

CO_2 Consumption of Technician's Subzero Storage Cabinet

Different trials were made to test the CO_2 consumption of the subzero cabinets. In summary it may be said that for the most efficient operation, the CO_2 should not be crushed except for the comparatively small portion which is kept in the alcohol. Even this portion of the CO_2 should be used in chunks as large as practical, since reducing the material to smaller pieces increases the amount of surface exposed and thus increases its rate of dissipation. The temperature of the alcohol can be maintained at −76° C. in this manner.

In order to find out how much of a problem an inseminator would experience in the use of this storage and carrying equipment, further tests were carried out, which determined that the subzero cabinet and a standard gallon Thermos jar used approximately 10 lb. of CO_2 per day. Of this total the Thermos jar consumed about 2½ lb. daily (Figure 4). In conjunction with these consumption findings, the trials proved that it was advantageous to add CO_2 twice daily to the Thermos jar rather than to replenish its supply only once. A nearly constant temperature of approximately −74° C. can be maintained with two fillings of CO_2 daily, but with a single filling there is a wide variation during the 24 hours.

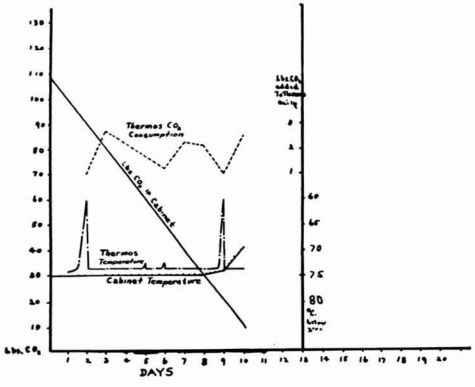

FIG. 4. CO_2 consumption of subzero cabinet in Trial III.

It is essential that some of the CO_2 be broken up and kept in the alcohol. Tests made without CO_2 in the alcohol gave varying temperature readings, which were stabilized when the CO_2 chunks were replaced. The slight extra use of CO_2 is more than offset by the even, exact temperature maintained with its use.

Under field conditions the CO_2 consumption of the technican's subzero storage cabinet should not vary greatly from the figure of 7½ to 8 lb. daily established by using solid blocks of CO_2 plus smaller pieces of the solid coolant placed directly in the alcohol.

Summary

A technician's subzero storage cabinet with a capacity of 135 lb. of CO_2 and 1,250 ampules of semen, or 100 lb. of CO_2 and 2,500 ampules, was constructed.

A low cost ampule-storage cartridge was also constructed to make the most efficient use of storage space within the subzero cabinet. The design of the cartridge is such that it may be used as a single unit in the gallon Thermos jar or in the technician's cabinet, or as a double unit for use in large central storage facilities. In any of these uses the contents of the cartridge and of the individual ampules can be easily identified.

It is essential to maintain a supply of CO_2 in the alcohol storage in order to maintain a constant storage temperature.

ACKNOWLEDGMENT

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Why Not A Leadership Training Program?

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In training college students it is customary to organize certain courses into a formal sequence, which is called a curriculum. Progress in these curricular subjects is measured at regular intervals and generally is the sole criterion for measuring scholastic advancement. However, in training for a profession, formal courses do not cover certain areas of training considered to be important in the development of potential industrial leaders.

In a survey of 26 leading corporations in this country, H. C. Hunt, principal of a Meriden, Conn., high school, found that only 10% of the individuals discharged lost their jobs because of lack of specific skills or technical knowledge, whereas 90% were discharged because of certain character traits. A lack of specific skills prevented 23.5% from getting promotions, and character traits prevented 76.5% from being advanced in their respective fields.

After 17 years of experience in the engineering profession, W. T. King of the General Electric Company concluded that the chief obstacles to the success of individual engineers were personal rather than technical. He found greater violations of the unwritten laws of personal conduct than of the written laws of science and stated that the unwritten laws are

recognized as important by all industrial leaders. Many more references could be cited showing the importance of personal traits in professional development.

Industry is now conducting the greatest manhunt in history for prospective leaders in different fields. The favorite hunting ground is on the campuses of the many colleges and universities. It is sometimes said that from 3 to 5% of all college students may be considered leaders. During their college training period this group frequently choose experiences which make them better leaders, but the vast majority of students graduate without a knowledge of what leadership is and have no idea how it can be developed.

There is no precise way to measure leadership, but a leader soon makes his presence felt by his contributions and conduct. A leader is a man who can get things done through others. This ability involves many things but, in general, is associated with the following characteristics.

The first is a talent for working effectively with others. This involves an individual's ability to present his ideas, to influence, "sell," persuade, and to secure the confidence and respect of other people. This important capacity depends on one's interest in, and understanding of, people and the ability to see the other fellow's point of view. It includes persuasiveness, tact, diplomacy, finesse, enthusiasm, self-confidence, impressiveness, sense of humor and perspective, according to the needs of the particular job. Skill in writing and speaking effectively is very important in this area.

The second characteristic is the ability to organize activities so that each individual can function most effectively. This involves building a first-class team of good men, well trained and well placed; looking ahead to size up a situation, crystallize objectives, formulate sound constructive policies and programs, and plan and schedule performance so that objectives will be achieved easily and harmoniously.

The third characteristic is the ability to recognize, think through, and work out sound solutions to problems. A man's value in this direction involves his keenness and intelligence; his analytical capacity; his ability to develop a conclusive case; his soundness, balance, objectivity; imagination, originality, breadth of vision and comprehension; and his intellectual honesty and courage.

The fourth essential quality involves a man's professional competence; his authoritative knowledge within a specific field. This ability rests upon an individual's natural aptitude, his academic training, his practical experience, and his zeal for continuing to widen his horizons to keep himself up-to-date. Of the above four areas important in the development of leadership, only the fourth is covered in formal academic courses; the others must be developed outside the classroom.

The most logical place to develop leadership is in student activities, and a student activity could have no more worth while objective than that of developing these qualities. The question that presents itself is how can such an objective be included in the program of an activity. It has already been indicated that the ability to associate harmoniously with other people is fundamental to the development of leadership. There is no better place to develop the "art" of human relations than in activities. By use of an anonymous questionnaire a member can get information about his personality from fellow members. This plus a sincere desire to improve will help much in an individual's development.

Since the ability to write and speak is important, every member should be given an opportunity to develop his talents. Writing committee reports and articles for news letters or year books, preparing minutes, and answering correspondence are all ordinary projects that can aid in this objective. Activities present an excellent opportunity to develop speaking ability by giving the individual an opportunity to present his ideas. Such a program will be most effective if activity leaders insist on high standards of performance—performance that requires previous preparation. The members can gain much valuable experience in speaking by giving reports and taking part in panel discussions and formal programs.

Activities present an excellent opportunity for an individual to reach objectives through others because the normal functions of such organizations are usually performed through committees. A student's first real opportunity to develop this ability comes when he is appointed to be a committee chairman. The chance to form a first class committee, to reach an objective, or perform a function, followed by a well written report, is an opportunity that should not be ignored. The committee chairman must size up the situation, crystallize objectives, formulate programs, and schedule performances that attain the desired objectives.

It is in activities that a student soon learns about the importance of good organization, for if it is lacking, nothing is accomplished. He has an opportunity to learn good business practices, such as budgeting and meeting a budget. The experience to be gained in the promotion of social functions and special projects through newspapers, journals, newsletters, and posters should be sought by every individual who would like to climb the ladder of leadership.

To develop an activity program that will be a firm stepping stone in the development of leadership in students may require an entire reevaluation of activity objectives. This reevaluation may stress objectives never before appreciated and thus inspire more vigorous effort.

Research Findings Reported by A. D. S. A. Workers

Summaries of papers presented at Eastern Division meeting

High Protein Grass by Fertilization

C. H. Ramage, R. E. Mather, C. Eby, and E. R. Purvis

Rutgers University

A large soil area located in southeastern New York, northern New Jersey, and western Connecticut is represented by Duchess shaly loam and related soil types—an area estimated to comprise some 3½ million acres. Because much of this soil is low and wet, or underlain with hardpan, alfalfa stands have an average life of only about 3 years.

In the fall of 1952, an experiment was established at the Dairy Research Farm at Sussex, N. J., to determine whether grasses under heavy nitrogen fertilization could approach alfalfa in yield and protein content. Grasses used in the experiment were orchard grass and reed canary grass. Nitrogen rates on the grasses were 50, 100, 200, and 400 lb. per acre per year with all plots receiving a basic fertilizer application of 100 lb. per acre of both potash and phosphoric acid.

First year yields of orchard grass for the four nitrogen treatments were 6,400, 6,850, 7,950, and 9,150 lb. of dry matter per acre. Second year yields were 4,250, 5,750, 6,950, and 8,900 lb. per acre. The reduction in yield of the low nitrogen plots from the first year to the second illustrates the rapid decline in production of grasses which do not receive adequate nitrogen. The per cent of crude protein in the orchard grass was increased by the treatments as follows: 50 lb. N—12.2%, 100 lb. N—13.6%, 200 lb. N—15.8%, 400 lb. N—20.1%. The yield and protein content of the reed canary grass followed a pattern very similar to that of orchard grass.

Two alfalfa-grass mixtures were seeded at the same time as the grasses, fertilized with 1,500 lb. per acre of 0-10-10, 0-10-20, 5-10-10, 5-10-20, 10-10-10, and 10-10-20. The second year, 1954, the best alfalfa-bromegrass treatment (0-10-20) yielded 7,800 lb. of dry matter having a protein content of 22.5%.

This experiment has indicated that it is possible, through use of adapted varieties of grasses and high rate of fertilizer, to produce forage equal to alfalfa in yield and protein content.

Further research is indicated on the following:

1. Feeding value of high protein grass forage.
2. Nitrate content of such forage, and its effect on livestock.
3. Supplemental feeding with such forage—perhaps the protein content of concentrates fed with high nitrogen forage could be very low.

Investigations with Come-Up-Time Pasteurization

R. B. Read, Jr., D. J. Hankinson, Warren Litsky, and N. L. Norcoss

University of Massachusetts

An apparatus has been constructed which will heat milk continuously by heat transfers from a small bore stainless steel tube, which in turn is heated by electric resistance. With a preheat temperature of 135° F., milk was heated to any desired temperature below the boiling point of milk. Heating times of 0.25 and 0.5 second with a holding time of less than 0.05 second were investigated to determine the effects of such a process on various milk properties.

Phosphatase destruction was found to occur at a mean of 182.4° and 178.5° F. for heating times of 0.25 and 0.5 second, respectively. Coagulation by rennin indicated that protein denaturation was not severe in this process. Flavor tests for heated flavor have been shown to be either the same or less than that of vat pasteurized milk. No significant changes in pH have been demonstrated in this process. Cream line formation was drastically reduced by temperatures over 85° C. The heating curve in this process was essentially linear.

The 99.9% destruction points for MS 102 were means of 88.8° and 88.1° C. for heating times of 0.25 and 0.5 second, respectively. *Corynebacterium diphtheriae* in concentrations of 1.2×10^6 per ml. was sterilized at mean temperatures of 88.9° and 87.1° C. for 0.25 and 0.5 second heating times, respectively. Concentrations of 5×10^6 per ml. of *Shigella paradysenteriae* were sterilized at mean temperatures of 72.7° and 72.2° C. for heating times of 0.25 and 0.5 second, respectively. *Streptococcus pyogenes* in concentrations of 3×10^6 per ml. were sterilized at mean temperatures of 72.6° and 70.2° C. with heating times of 0.25 and 0.5 second, respectively. *Salmonella typhosa* in concentrations of 3×10^6 was sterilized at mean temperatures of 74.0° and 72.6° C. for heating times of 0.25 and 0.5 second, respectively. *Escherichia coli* was sterilized at mean temperatures of 79.2° and 78.0° C. for heating times of 0.25 and 0.5 second, respectively, with an initial concentration of 8×10^6 cells per ml. An investigation using *Mycobacterium tuberculosis* is being carried out at the present time.

Animal and Dairy Husbandry Curricula in the Land-Grant Colleges

R. C. Foley and F. F. Michelson
University of Massachusetts

A detailed study of animal and dairy husbandry curricula in the land-grand colleges based upon the latest catalogs available in the University of Massachusetts Library revealed many interesting facets of this problem facing all teachers and administrators in these fields. The study emphasized the wide variation that exists among the 48 institutions in their prescribed curricula. Based upon credit hours required for graduation, the average student spent his time as follows:

12%—liberal arts
23%—basic science
18%—major courses
18%—supporting agricultural courses
29%—free electives

The mimeographed summary contains seven tables giving detailed course requirements for animal husbandry and dairy husbandry majors, other required courses in the School or College of Agriculture, in liberal arts and the basic sciences. The authors do not attempt to prescribe an ideal curriculum for animal or dairy husbandry majors but emphasize the need for a broad, well-balanced, undergraduate curriculum and for continuing study and revision if undergraduate majors are to receive the best possible training for future careers in agriculture.

The Relative Merits of Five Measures of a Dairy Sire's Transmitting Ability

S. N. Gaunt
University of Massachusetts
and
J. E. Legates
North Carolina State College

The five measures studied were the daughter average, daughter-dam difference, equal parent index, daughter-contemporary herd difference, and daughter-contemporary herd index.

Analyses were made on DHIA sire proofs published for the years 1949-51 of 836 Guernsey and Holstein sires used in artificial breeding associations in the United States. Production records for milk, per cent test, and butterfat were analyzed of 6,949 daughters, 6,201 different dams, and the contemporary herd averages of 2,420 different herds. Naturally and artificially sired daughters were analyzed separately.

The estimated herd variances accounted for 28.1 to 37.6% of the total variance of a single butterfat record, the sire variances for 4.4 to 10.2%, and the error (residual) term variances for 52.3 to 62.2%. Based on the repeatability estimates using the variance components in regression formulas, no one measure was found to be superior. However, it is indicated that the daughter-contemporary herd difference or herd index has much usefulness as a corrector of some of the environmental differences, particularly where there are large differences between herds.

CALL FOR PAPERS FOR THE 1956 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The 51st annual meeting of the American Dairy Science Association will be held June 19, 20, and 21, 1956, at the University of Connecticut, Storrs.

Members who wish to present papers must furnish titles and abstracts not later than March 1. Two copies of each abstract including the original must be mailed to the Chairman, another to the Vice-Chairman, and a fourth one to the Secretary of the Section before which the paper will be given. Abstracts must not contain over 200 words. Abstracts longer than 200 words will be returned to the authors by the committee. Terminology should be understandable by the general reader.

It is essential that titles and abstracts be received by March 1 so that the complete program may appear in the Journal of Dairy Science for May and so that the abstracts may be printed for distribution at the annual meeting. If time limitations make it necessary, some papers may have to be read by title only. No member should plan to present more than one paper.

Reactions of those attending previous meetings indicate that papers presented by members in industry and by senior members of the Association are especially well received.

Members prefer to receive mimeographed copies of essential material which must be presented visually; such material includes tables of data, graphs, and brief summaries. Material for projection on screens should be used only if the preferred method is impossible.

Names and addresses of officers of sections to whom titles and abstracts will be sent are:

EXTENSION SECTION

Chairman: G. M. WERNER, Dairy Husbandry Department, University of Wisconsin, Madison.

Vice-Chairman: JAMES BURKE, Animal Husbandry Department, Ithaca, New York.

Secretary: LEO FRYMAN, Department of Dairy Science, University of Illinois, Urbana.

MANUFACTURING SECTION

Chairman: W. M. ROBERTS, Department of Animal Industry, North Carolina State College, Raleigh.

Vice-Chairman: H. L. TEMPLETON, 6125 Florence Blvd., Omaha, Nebraska.

Secretary: F. J. BABEL, Department of Dairy Husbandry, Purdue University, Lafayette, Indiana.

PRODUCTION SECTION

Chairman: N. P. RALSTON, Dairy Department, Michigan State College, East Lansing.

Vice-Chairman: S. W. MEAD, Department of Animal Husbandry, University of California, Davis.

Secretary: S. B. MARSHALL, Department of Dairy Science, University of Florida, Gainesville.

AUTHOR INDEX OF ORIGINAL ARTICLES

* Abstract of paper presented at the annual meeting.

** Abstract of paper presented at Eastern Division meeting.

* Abstract of paper presented at the annual meeting.

** Abstract of paper presented at Eastern Division meeting.

AUTHOR INDEX OF ABSTRACTS

JOURNAL
OF
DAIRY SCIENCE

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JANUARY—DECEMBER 1955

1955
THE AMERICAN DAIRY SCIENCE ASSOCIATION

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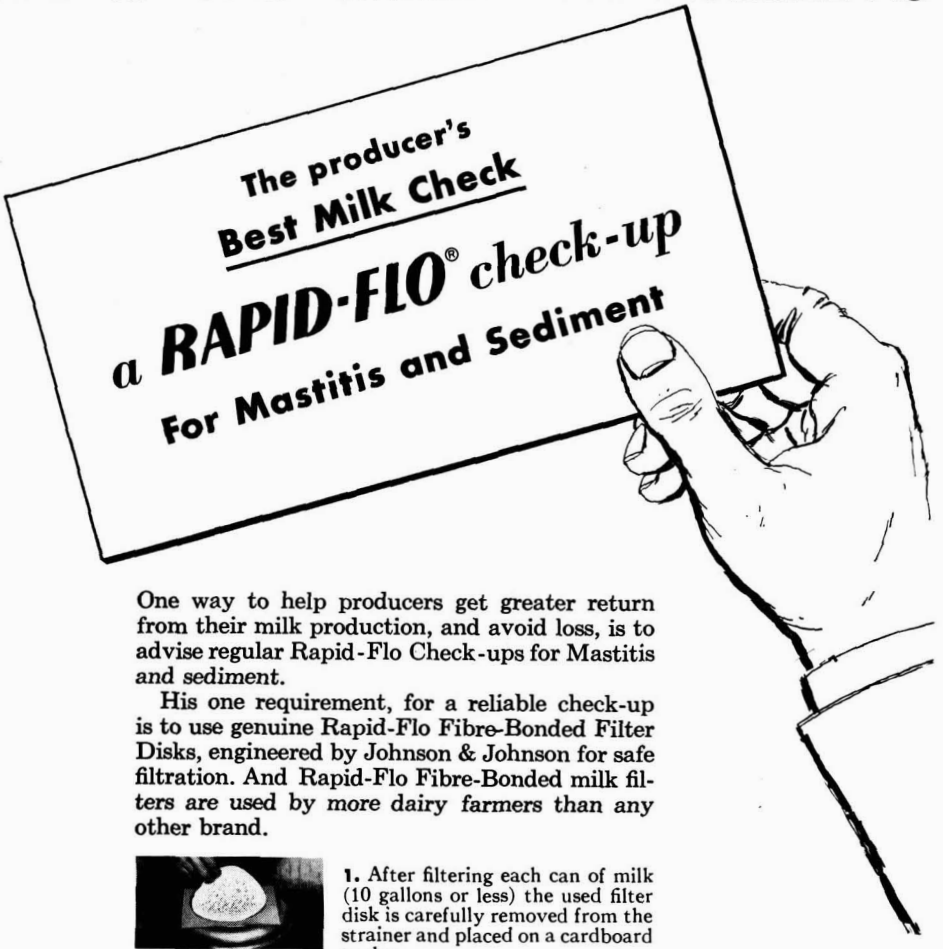
TIPS TO SANITARIANS



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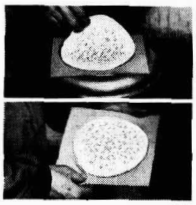

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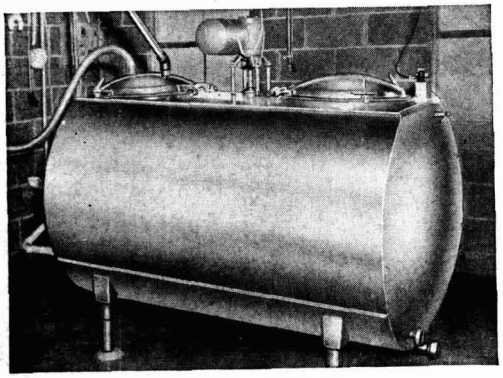

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The above media are also supplied in packages of 12 tubes of any one medium. In addition, for more detailed determinations, ATS Sensitivity Media and Lowenstein Sensitivity Media containing Streptomycin 250 mcg., Viomycin 250 mcg., and INH 0.2 mcg. are available in packages of 12 tubes.

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