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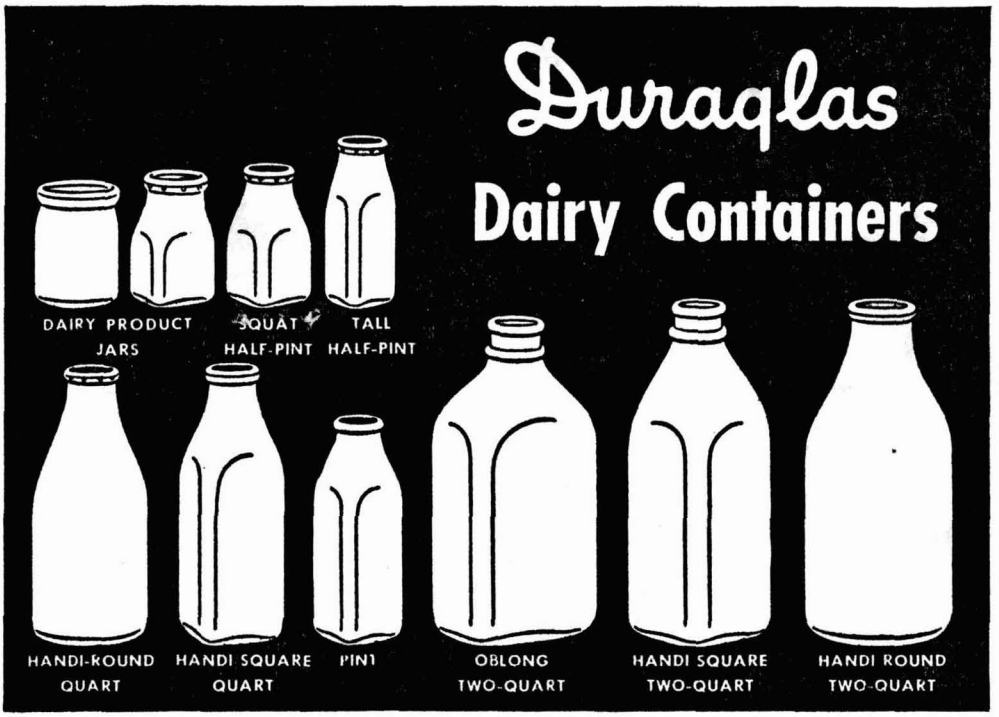

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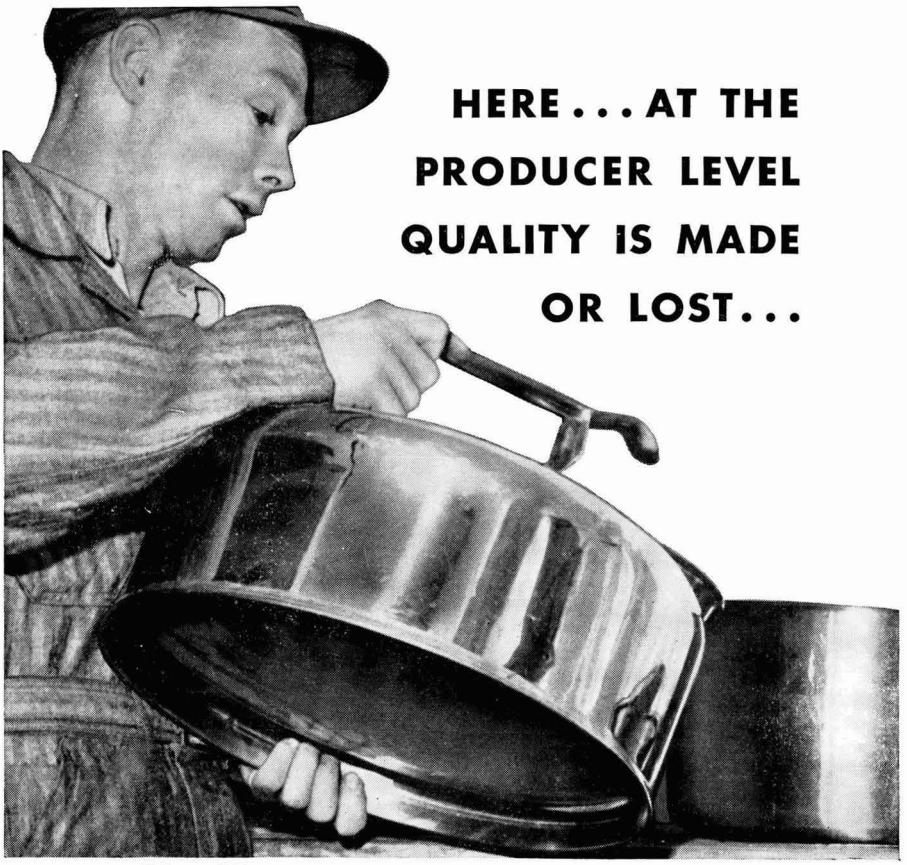

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VOLUME XXXV AUGUST, 1952 NUMBER 8

THE INTERRELATIONSHIPS OF PROCESSING TREATMENTS AND OXIDATION–REDUCTION SYSTEMS AS FACTORS AFFECTING THE KEEPING QUALITY OF DRY WHOLE MILK[1, 2]

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The beneficial effect of heating fluid milk in excess of normal pasteurization on the resistance of the resulting dry whole milk to the development of tallowy flavor was first demonstrated by Holm *et al.* (15). This effect has been corroborated by many other workers, as indicated in a recent review (6). It usually is attributed to liberation of highly active sulfhydryl groups from the milk proteins by the treatment (10, 16, 6), but this explanation has not been proved by adequate quantitative data.

The sulfhydryl groups of proteins exhibit a graded order of reactivity and consequently the sulfhydryl titers of raw and heated milks depend on the reagent employed. Thus, the relatively inactive groups of raw milk do not react with nitroprusside or thiamine disulfide (12, 16) but can be titrated by the *o*-iodosobenzoate-iodine method of Larson and Jenness (19, 20). Heat denaturation increases the activity of protein sulfhydryl groups as exemplified, in the case of milk, by positive nitroprusside and thiamine disulfide tests. In fact, some groups are activated sufficiently to be oxidizable by atmospheric oxygen. Thus, a low oxygen tension during and following heating preserves the active reducing substances (13, 20). This preservation of reducing groups has been advanced as an explanation for the superior keeping quality imparted to dry whole milk by deaeration before heating (11).

Reducing substances produced in milk due to sugar-protein interaction during processing must be considered in evaluating the redox systems of dry whole milk. These substances together with ascorbic acid and certain of the sulfhydryl groups are included in the reducing capacity as measured by the modified acid ferricyanide method of Crowe *et al.* (2). Very little over-all change in the acid ferricyanide-reducing substances (AFRS) occurs during preheating, but large increases may occur during drying and storage (5, 2). Harland *et al.* (13) reported that the keeping quality of dry whole milk specially processed to obtain a high content of AFRS was not superior to normally processed milks.

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[1] Paper no. 2802, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Data presented in this paper are taken from a thesis by H. A. Harland in partial fulfillment of requirements for the Ph.D. degree, University of Minnesota, 1950.

Although ascorbic acid is an important reducing constituent of milk and has been suggested as a key link in the lipide oxidation of fluid milk (17, 18), its role in oxidative deterioration of dry whole milk is questionable (3, 22). In any event, small variations in the ascorbic acid content do not greatly affect the keeping quality of the dry milk.

Some attempts have been made to associate the oxidation-reduction systems of milk as measured by redox potentials with its resistance to oxidation. Greenbank and Wright (11) demonstrated that deaeration before preheating lowered the Eh and improved the keeping quality of the dry whole milk. More recently, Decker and Ashworth (7) found that the degree of off-flavor development was associated with an increase in the Eh during storage of dry whole milk.

The following experiments were designed to obtain more information concerning the factors influencing the effectiveness of heat treatment of fluid milk on the antioxygenic properties of the resulting dry whole milk and to investigate several objective tests that appeared to afford some promise for evaluating the heat treatment of milk for drying purposes.

METHODS

The oxidation-reduction potential of the milk was determined at 30° C. with a Leeds and Northrup, type K, potentiometer and a saturated calomel half-cell. The sample was contained in a 150-ml., wide-mouth, extraction flask fitted with a rubber stopper holding three bright platinum electrodes, each of which could be connected to the calomel half-cell by a KCl-agar bridge. In routine determinations, the potentials were recorded at 30-min. intervals until they remained practically constant. The use of multiple electrodes compensated for polarization effects.

Thiamine disulfide-reducing substances (TDRS) were determined essentially according to the method of Harland and Ashworth (12). Acid ferricyanide-reducing substances (AFRS) were estimated according to the Chapman and McFarlane (1) method as modified by Crowe *et al.* (2). Iodosobenzoate-reducing substances (IBRS) were determined according to the procedure outlined by Larson and Jenness (19, 20). Ascorbic acid, which is oxidized by this reagent, was subtracted from IBRS titers. Ascorbic acid content of the milk was estimated as suggested by Doan and Josephson (9). Fat peroxide values of the dry milks were determined according to the recommendations of Hills and Thiel (14). Flavor scoring of the milk was done by three experienced judges in accordance with the student score card. The dry whole milks were stored in no. 200 tin cans at 37° C.; part of each lot was packed in air and part in nitrogen.

EXPERIMENTAL

A. Effect of processing on some of the oxidation-reduction systems of milk.

Influence of oxygen tension during heating. Evening milk was transferred from a stainless steel milking machine bucket to a Pyrex glass container, saturated with toluene, cooled in an ice bath and stored at 5° C. until the following day when the samples were given a 30-min. holder pasteurization at 85° C. in the 150-ml. flasks used for the Eh determination.

The effect of oxygen tension on some of the redox systems was observed with milk in equilibrium with air, oxygen and nitrogen. The treatments in oxygen and nitrogen consisted of continuous bubbling of a slow stream of the gas through the sample from the time of placing it in the bath for the initial Eh measurements until the heat treatment and subsequent Eh measurements were completed.

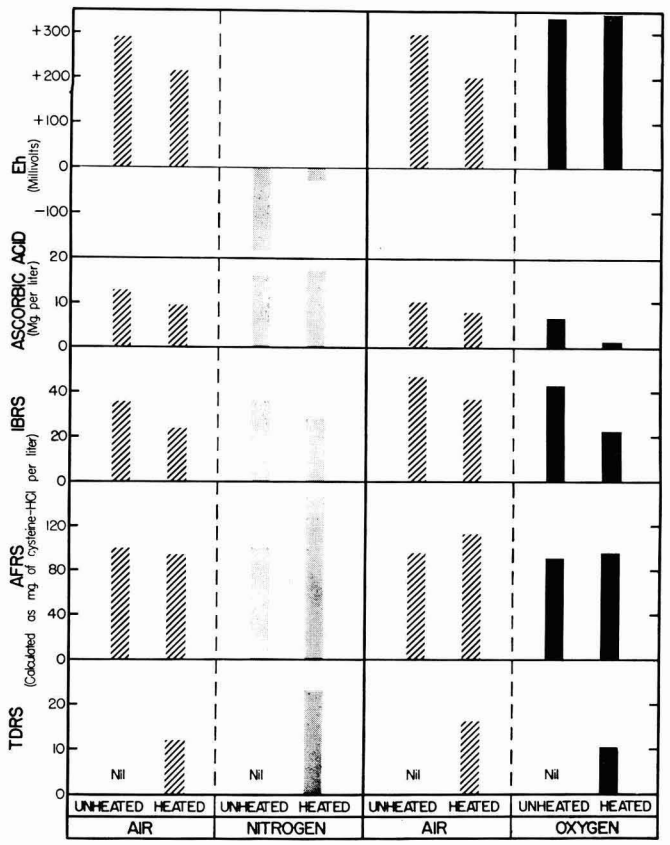

FIG. 1. The effect of oxygen on some of the oxidation-reduction systems of milk heated at 85° C. (a) The comparison of one lot of milk in equilibrium with air with this milk after equilibration with nitrogen and (b) the comparison of a second lot of milk also in equilibrium with air with this milk after equilibration with oxygen.

The results of one of a duplicate series of experiments are illustrated in figure 1. The marked reduction in the Eh of the milk heated in equilibrium with nitrogen as compared to oxygen is reflected in the higher values for the content of ascorbic acid, IBRS and TDRS. The AFRS, which represent a composite of these and other reducing systems, also show higher reducing values for milk heated in the absence of oxygen.

Influence of time and temperature of heating and the effects of condensing

and drying. The milk used for this work was taken from a grade A supply of raw milk used by the University bottling plant. Six lots were subjected to 30-min. holder pasteurization, two at each of three temperatures, 76.7° C., 85° C. and 96° C. Ten more lots were subjected to various short-time heat treatments in a laboratory-constructed continuous heater consisting of three sections ((a) forewarming, (b) heating and (c) holding) of glass tubing immersed in a circulating water bath. The heating time was regulated by the volume of the holding section and the rate of flow of the milk through the apparatus. The pasteurization treatments of all 16 lots of milk are indicated in table 1. The heated milks were condensed to approximately 35 per cent solids in an all-glass vacuum distillation apparatus and dried in a laboratory spray drier designed by Coulter (4).

TABLE 1

The heat treatments given the fluid milks used for the preparation of 16 samples of dry whole milk

Sample no.	Heat treatments		Sample no.	Heat treatments	
	Temp.	Time		Temp.	Time
	(° C.)			(° C.)	
1	85.0	25 sec.	9	90.0	80 sec.
2	85.0	37 sec.	10	96.0	60 sec.
3	85.0	47 sec.	11	96.0	74 sec.
4	96.0	15 sec.	12	96.0	78 sec.
5	96.0	13 sec.	13	85.0	30 min.
6	85.0	74 sec.	14	85.0	30 min.
7	76.7	30 min.	15	96.0	30 min.
8	76.7	30 min.	16	96.0	30 min.

The influence of heat treatment and drying on some of the redox systems of milk are shown in figure 2. These data, with the exception of the 60-sec. treatment at 96° C., which represents a single lot, are average values obtained for two lots of fluid milk. Since condensing has little effect on the oxidation-reduction systems, these data have been omitted. With the exception of AFRS, the data in figure 2 clearly demonstrate the oxidative influence of both 30-min. preheating and drying on the redox systems of milk. The values indicated for the AFRS are influenced not only by the TDRS, IBRS and ascorbic acid but also by the lactose-protein interaction that occurs during drying of the condensed milk. The short-time heat treatments at 96° C. resulted in comparatively small losses in both ascorbic acid and iodosobenzoate titers and in greater contents of TDRS.

B. The effect of storage on the quality and certain of the oxidation-reduction systems of dry whole milk.

The 16 lots of dry whole milk described in the preceding section were stored in air and nitrogen packs at 37° C. Some of the effects of storage on ten of these lots are shown in figures 3 and 4. For the sake of clarity, the data for six of the lots which received intermediate treatments were omitted from the graphs.

In air pack the dry samples prepared from fluid milks preheated at 96° C. for about 76 sec. (lots 11 and 12) exhibited the best keeping quality and those that received the 30-min. treatments at 85° C. (lots 13 and 14) the poorest keeping

quality of the 16 lots studied. However, this difference in keeping quality was largely nullified by nitrogen packing.

Storage of the dry whole milk for 3 mo. in air pack resulted in no change in the original content of TDRS or IBRS but in significant losses of ascorbic acid and increases in the AFRS. The loss in ascorbic acid was reflected in an increase in the redox potential during air storage. The data further show that,

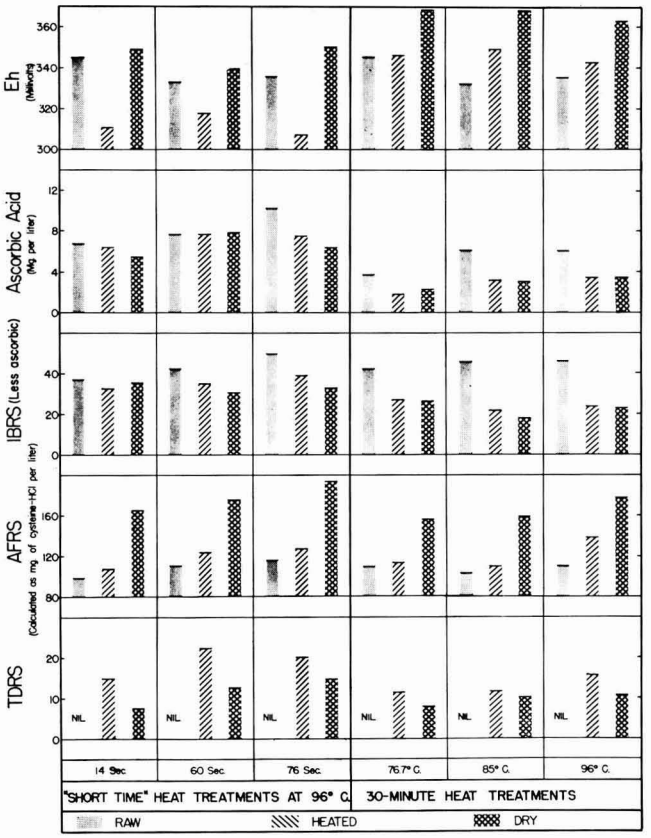

FIG. 2. The effects of processing on some of the oxidation-reduction systems of milk. Short-time heat treatments at 96° C. are compared with 30-min. treatments.

although the Eh of the dry milk may increase somewhat during storage, the relative differences among samples are maintained.

C. Some interrelationships of the keeping quality and the oxidation-reduction system.

That the production of fat peroxides may be a major factor in the flavor deterioration of dry whole milk during storage in air is demonstrated in figure 5. The correlation coefficient of +0.75 (significant at the 1 per cent level) is espe-

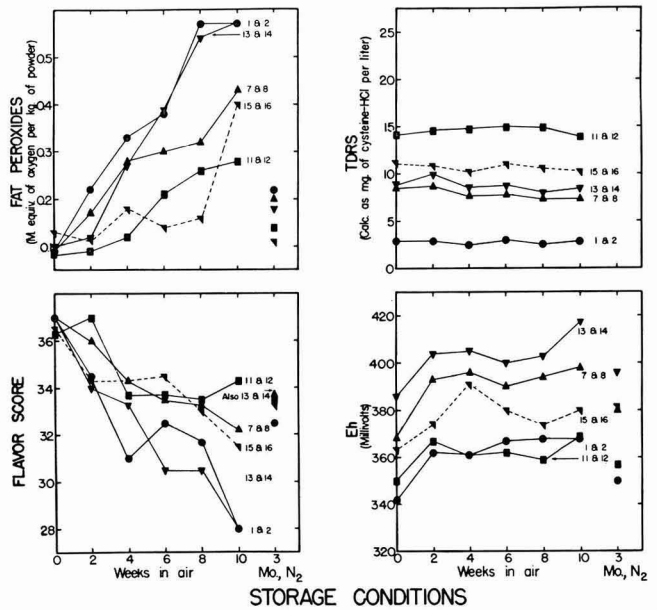

FIG. 3. The influence of storage of 10 lots of dry whole milk in air and in nitrogen at 37° C. on the flavor score, Eh and on the content of TDRS and fat peroxides.

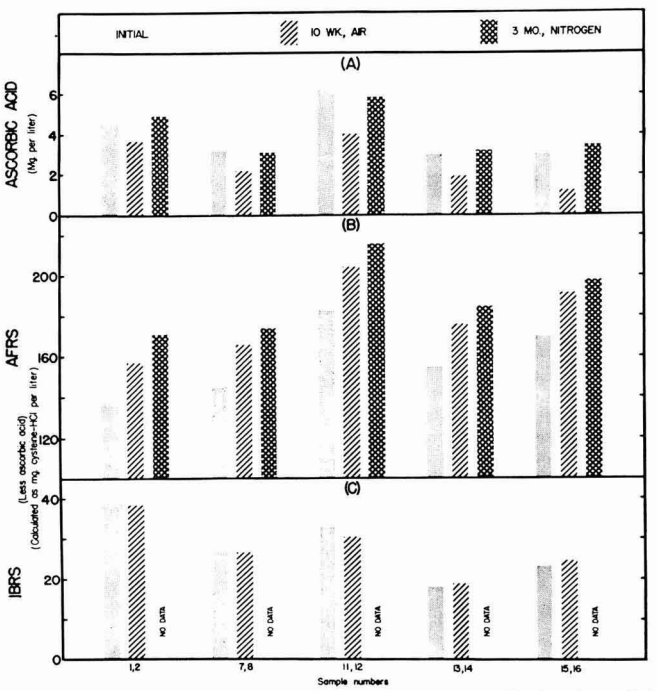

FIG. 4. The influence of storage on 10 lots of dry whole milk in air and in nitrogen at 37° C. on the content of ascorbic acid, AFRS and IBRS.

cially high in view of the fact that there are other causes for loss in flavor score besides those associated with fat peroxide formation.

Some of the relationships of the IBRS, AFRS and Eh to the flavor deterioration of the 16 samples of dry whole milk during storage are shown in figure 6. There is a negative correlation (significant at the 1 per cent level) between the content of TDRS and the loss in flavor score and the increase in fat peroxides (fig. 6 A, B), the relationship being especially close ($r = -0.88$) between the TDRS content and the loss in flavor score of the milk preheated for less than 90 sec. It is equally important that there is no relationship between the content of TDRS and the keeping quality of those milks subjected to 30-min. preheating.

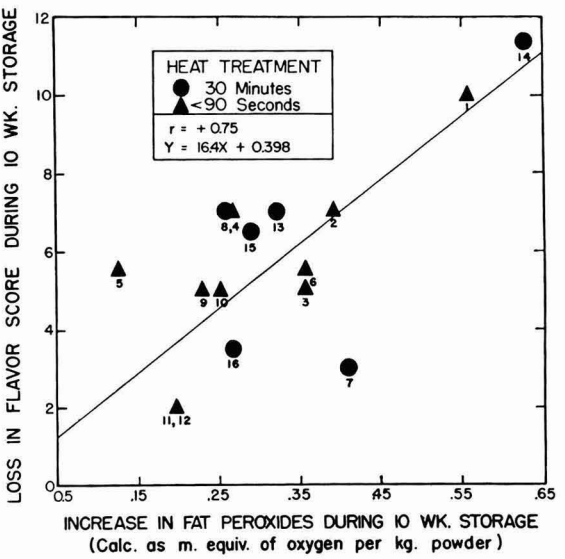

FIG. 5. The relationship of the increase in fat peroxides to the loss in flavor score of 16 lots of dry whole milk during 10 wk. of storage in air at 37° C.

The curvilinear relationship of the content of AFRS to the increase in the fat peroxides during aging of the milk (fig. 6C) is of only limited usefulness, since milk of relatively low peroxide content following storage may have initial contents of AFRS covering the entire normal range of these substances. Furthermore, low AFRS values may be associated with powders having either good or poor keeping quality, but a high initial content of AFRS may indicate good resistance of the dry milk to oxidation.

There was no apparent relationship between the initial Eh and the loss in flavor score during storage of the 16 lots of dry whole milk (fig. 6D). The Eh values are influenced by relatively small variations in the ascorbic acid content, as indicated in figure 7. There was no relationship between either the initial content or loss in ascorbic acid and the loss in flavor score of these samples of air-packed dry whole milk during 10 wk. at 37° C.

DISCUSSION

Heat treatment of fluid milk for drying purposes usually has three objectives: (a) Destruction of pathogenic bacteria; (b) destruction of undesirable enzymes; and (c) production of antioxygenic substances, generally considered to be "free" sulfhydryl groups. This paper has been concerned largely with the last objective.

Deaeration of raw fluid milk was found to decrease the Eh of the system over 400 mv., which corroborates the data of Saal and Heukelom (21). Furthermore,

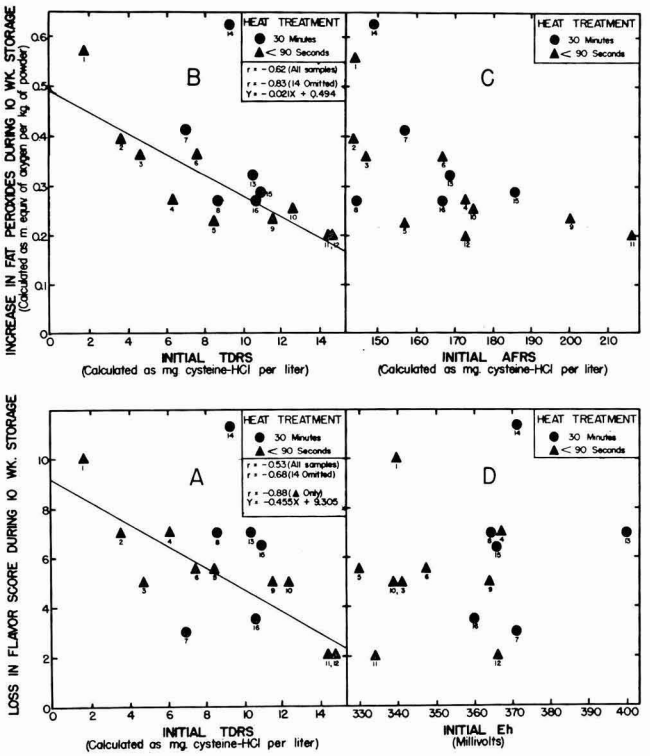

FIG. 6. The relationships of (A) initial TDRS and (D) initial Eh to loss in flavor score and of (B) initial TDRS and (C) initial AFRS to increase in fat peroxides during 10 wk. of storage of dry whole milk at 37° C.

the Eh value of milk subjected to heat treatment following deaeration was found to be much lower than that of milk given like treatment in air. This result also was secured by Greenbank and Wright (11), who attributed the lower Eh value to heat-induced reducing substances. It was shown earlier by Harland *et al.* (13) and by Larson and Jenness (20) that deaeration previous to heat treatment causes improved retention of heat-induced reducing substances. Although deaerated, preheated milk was not condensed and dried in the present work, Greenbank and Wright (11) found that dried milk prepared in this way had

greater resistance to oxidation during storage than milk processed in the usual manner.

High-temperature, short-time (HTST) treatment (90 to 96° C. for 60 to 80 sec.) imparted greater reducing properties to fluid milk than holder treatment (76.7 to 96° C. for 30 min.) due to more rapid production of reducing groups and less time for oxidation. This is evidenced by lower Eh values, less oxidation of ascorbic acid and greater retention of iodosobenzoate and thiamine disulfide-reducing substances. Dry milk prepared from HTST heated milk also had greater reducing capacity, although there was a certain amount of oxidation during the drying process.

The relationship of the Eh of fluid or reconstituted milk to the resistance of dry milk to oxidation is a controversial question (11, 7). In the present work,

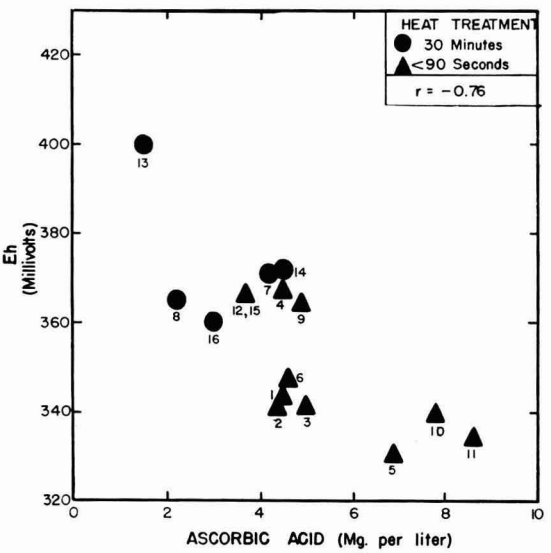

FIG. 7. The relationship of the initial ascorbic acid to the initial Eh of dry whole milk.

the Eh values of milk reconstituted from freshly manufactured dry milk lay within the comparatively narrow range of 340 to 390 mv. and were of little value for predicting the keeping quality of the dry product. The Eh of the dry milk reconstituted after 10 wk. of storage in air at 37° C. was characteristic of the original value, which in turn was largely determined by the ascorbic acid content (actually the ratio of ascorbic acid to dehydroascorbic acid). Small variations in ascorbic acid content had a relatively large effect on the Eh (see also 21) but similar variations had almost no effect on keeping quality.

An attempt has been made to relate the keeping quality of dry whole milk with each of several components of the oxidation-reduction system. With HTST preheated samples there is a close relationship (correlation coefficient, −0.88) between the content of TDRS and the loss in flavor score during 10 wk. of storing

dry whole milk in air at 37° C. Such a relationship does not hold for those samples prepared from milk preheated for 30 min., probably because some of the free –SH groups are oxidized and other oxidative processes occur during the prolonged heat treatment. Although there are no data on this point, maximum TDRS values and likewise maximum resistance to oxidation during storage should be expected to dry whole milk prepared from milk deaerated prior to HTST preheating. Decker *et al.* (8), using 20-min. treatments at 150, 160 and 170° F., suggested that the destruction of an oxidative enzyme system may explain the superior keeping quality of dry whole milk that had been preheated at 170° F. In the present study, the definite superiority of short-time preheating to 30-min. treatments does not appear to support enzyme destruction as an explanation of the beneficial effects of preheating in dry milk manufacture.

The content of IBRS may not be used to predict the keeping quality of dry whole milk, but determination of these substances can be of value when considered together with TDRS. For example, two lots of milk, one short-time and the other long-time heat-treated in air may have similar contents of TDRS, but the iodosobenzoate titre of the short-time treated milk could be expected to be higher. However, there is one important condition to the use of IBRS in this manner. Since the iodosobenzoate titre continues to decrease for some time following heat treatment of milk in the presence of air or until the milk is dried, (20), any discontinuity in the processing might make interpretation of the results difficult.

The AFRS (exclusive of ascorbic acid) occupy a unique position in the redox system of milk. A major portion of these reducing substances in dry milk is contributed by products of lactose-protein interaction and, being very weak reducing agents, they have little, if any, influence on the redox potential. Although the AFRS increase greatly during the drying process and slowly during storage, the over-all change during drying and storage is oxidative, as indicated by the tendency for the Eh to increase.

SUMMARY

High-temperature-short-time treatments (90 to 96° C. for 60 to 80 sec.) are superior to 30 min. at 76.7 to 96° C. for preheating fluid milk for drying purposes. The short-time preheating results in lower oxidation-reduction potentials, less oxidation of ascorbic acid and greater retention of iodosobenzoate and thiamine disulfide-reducing substances in both the fluid and dry product. Furthermore, dry whole milk prepared with the short-time preheating exhibits better resistance to oxidation during storage.

The removal of oxygen from the system previous to a 30-min. heat treatment at 85° C. results in lower Eh values, better retention of thiamine disulfide-, acid ferricyanide- and iodosobenzoate-reducing substances than if the heating is done in air or in equilibrium with oxygen.

There is a close relationship between the content of thiamine disulfide-reducing substances and the resistance of dry whole milk to oxidation during storage in air if HTST preheating is used.

The initial redox potential, the content of ascorbic acid, acid ferricyanide-

(AFRS), thiamine disulfide- (TDRS) or iodosobenzoate-reducing substances (IBRS) used individually are not reliable indices of the resistance of dry whole milk to oxidation. The estimation of both the IBRS and TDRS contents of fresh samples may be of value in estimating the storage life of dry whole milk.

ACKNOWLEDGMENTS

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The support of the Minnesota Institute of Research also is gratefully acknowledged.

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PREPARATION OF MILK FAT.[1] II. A NEW METHOD OF MANUFACTURING BUTTEROIL[2]

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A previous paper has reported the usefulness of certain simple organic compounds in de-emulsifying cream to recover milk fat in good yields (4). As a natural outgrowth of this and other work (5, 6), the possibility of adapting the de-emulsification procedure to the manufacture of butteroil was considered. Of the several widely used methods of preparation, the oldest is the "boiling-off" process described by El-Rafey *et al.* (1). This involves heating butter in an open kettle until all water present has been evaporated. Any non-fat solids precipitate as a brown sediment and this is strained from the oil. Most modern procedures for the production of butteroil are essentially centrifugal methods, in which butter is melted, the oil floated from any curd present and the oil-curd mixture remaining centrifuged in order to recover oil that might otherwise be lost in the curd. Regardless of procedure, these methods depend on the use of butter, a raw material which requires considerable time and expense to produce.

In the American method (7), butteroil is processed directly from cream through the use of a specially constructed separator which yields an oil of 90 to 95 per cent fat. This product is reseparated, heated under vacuum in a vacreator, steam-distilled and then cooled in a vacuum chamber. The usefulness of this latter procedure is limited considerably by the complicated processing and expensive equipment required. Accordingly, the object of this investigation was to study the practicability of using the de-emulsification principle in the manufacture of butteroil.

EXPERIMENTAL

In order to select those surface-active agents which might have best possibilities for large-scale use, the agents first were submitted to a laboratory de-emulsification test as follows: 10-g. samples of fresh, raw 40 to 45 per cent cream were placed in 15-ml. graduated conical centrifuge tubes. Various amounts up to 1 g. of the agent to be tested then were added. The agent and sample were mixed and placed in a hot water bath (180° F.) for 15 min. Following centrifuging, the tubes were tempered in a hot water bath (140° F.) for 5 min. and the volume of the oil layer observed.

Ninety-seven commercially available surface-active agents were submitted to the laboratory de-emulsification test. For the sake of brevity, those agents which gave negative results have not been enumerated.[3] The agents were tested in the

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[3] This information is available on request.

following three groups: (1) Twenty-five agents, representing many different types, were tested as received from their manufacturers; (2) the 25 agents of group 1 plus 55 additional agents were tested as 33 per cent by weight solutions or dispersions in butyl carbitol; (3) seventeen Span- and Tween-type agents were tested as received from their manufacturers and also as 25 per cent solutions or dispersions in ethanol. In group 1, the following were found to be effective at a concentration of 10 per cent or less: Tergitol 7 (T-7), Tergitol-4, Tergitol P-28, Ahcowet RS and Nopco 1392. In addition to these, 18 agents of group 2 gave quantitative de-emulsification at a level of 6 per cent or less by weight of their butyl carbitol solutions. These were: Antarane T-120, Aerosol OT, Phi O Sol WA, Victawet 35B, Ahcowet ANS, Duofol L, Bozetol, Arylene 1355, Hymolon K,

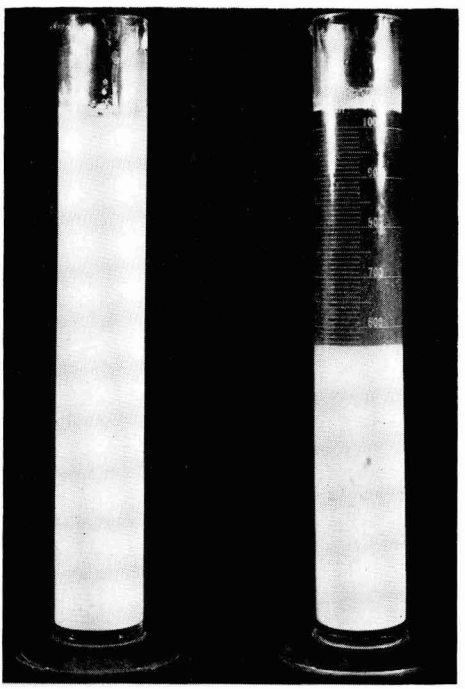

FIG. 1. De-emulsification of cream (40% fat) heated at 180° F. for 15 min. in 1-l. graduate. Left—control, right—with 3% Tergitol 7 added before heating.

Nonisol 200, Sulfanol KB, Warcosan, Warcosal 60-S, Sulfanole KB 40, Aerosol MA, Tamol N, Antarox A 480 and Nacconal NR. Under the conditions employed, none of the agents in group 3 promoted quantitative de-emulsification of cream at a level of 10 per cent or less. The action of many of the effective agents was striking. A clear, quantitative oil layer was observed frequently after only a few minutes in the hot water bath (fig. 1).

Laboratory and pilot-scale experiments were conducted with T-7 to study the variables of agent concentration, fat content of cream and heat treatment, as well

as adaptability of the procedure to large-scale operation. Time has not permitted a critical appraisal of all the effective agents; therefore, T-7 is not necessarily the most effective or most practical agent to use. However, results obtained with it are indicative of the possibilities inherent in the de-emulsification method.

Data in table 1 demonstrate that concentrations of T-7 ranging from 3.3 to

TABLE 1

Effect of various quantities of Tergitol 7 on the amount of oiling off produced in 9-g. samples of cream (44.5% fat)

T-7 added		Oil layer	Yield of butteroil[a]
(*ml.*)	(%)	(*ml.*)	(%)
0.0	0.0	0.0	0
0.1	1.1	0.3	7
0.2	2.2	1.6	36
0.3	3.3	4.3	97
0.4	4.4	4.3	97
0.5	5.6	4.3	97
0.75	8.3	4.3	97
1.0	11.1	4.3	97

[a] Based on pure butterfat having a density of 0.9 at 140° F.

11.1 per cent are equally effective in de-emulsifying cream (44.5 per cent) when the cream-agent mixture is held at 180° F. for 15 min. Concentrations below 3.3 per cent usually did not promote complete oiling-off. Data concerning the relationship of fat content of cream to the amount of T-7 required for effective de-emulsification are presented in table 2. Raw cream was standardized to

TABLE 2

Minimum concentration of Tergitol 7 required to promote complete de-emulsification in creams of various fat contents

Fat content of creams	Minimum concentrations effective	
(%)	(*ml./9 g. cream*)	(%)
41.0	0.2–0.4	2.0– 4.5
35.5	0.3–0.4	3.0– 4.5
31.0	0.4–0.5	4.5– 5.5
25.0	0.5–0.6	5.5– 7.0
20.5	0.6–0.7	6.5– 8.0
16.5	0.7–0.9	7.5–10.0
11.0	0.7–0.9	7.5–10.0
6.2	0.6–0.8	6.5– 9.0
3.5	0.7–0.8	7.5– 9.0

various fat contents ranging from 41 to 3.5 per cent, using raw skimmilk. These data indicate quite clearly that as the fat content of the cream is reduced, increasing quantities of T-7 are required for complete de-emulsification. Table 3 presents data concerning the influence of heat treatment on the de-emulsification process. The effectiveness of T-7 in de-emulsifying cream is influenced by the amount of heat treatment the cream-agent mixture receives. Much lower con-

centrations of T-7 were sufficient to obtain quantitative yields of butteroil at holding temperatures between 150 and 200° F. than at lower temperatures. Although slightly higher yields were obtained by using a holding temperature of 200° F., the oil layer was much clearer when 180° F. was employed. Use of 180° F. rather than some temperature between 150 and 180° F. seems recommended in order to provide a margin of safety in the method.

Pilot operations. The simplicity of the method and efficiency with which the Tergitols promoted de-emulsification in the preliminary trials with small samples of cream suggested that larger quantities of cream should be processed by the method to determine its commercial feasibility. Accordingly, several trials were conducted, employing approximately 75 lb. of cream as the starting material and T-7 as the de-emulsifying agent. The following procedure and results are representative: To one can of fresh raw cream, weighing 72.5 lb. and testing

TABLE 3

Effect of 15 min. holding temperatures on the capacity of Tergitol 7 to de-emulsify 9-g. samples of cream (40% fat)

T-7 added		% Yields of butteroil at temperatures of:					
		110° F.	130° F.	150° F.	170° F.	180° F.	200° F.
(*ml.*)	(%)						
0.1	1.1	.....	.....	.....	.....	7	.....
0.2	2.2	3	10	.....	.....	18	23
0.3	3.3	8	15	33	43	47	68
0.4	4.4	13	.....	100	100	100	99
0.5	5.6	13	85	100	100	100	.....
0.6	6.7	.....	80	100	97	.....	100
0.7	7.8	.....	90	.....	.....	.....	100
0.75	8.3	60	.....	.....	.....	92	.....
0.8	8.9	.....	95	.....	.....	.....	100
1.0	11.1	97	97	.....	.....	94	100
2.0	22.2	.....	.....	.....	.....	76	.....

43.5 per cent fat, were added with gentle agitation, 2.5 lb. of T-7. The mixture was heated in a hot water bath to 180° F. and held at that temperature for 15 min., during which time it was agitated continuously. The de-emulsified cream then was allowed to stand for approximately 15 min. after which the lower serum layer was removed by siphoning and replaced with an equal volume of hot water. The water-oil mixture was thoroughly agitated for a period of about 5 min. and then passed through a cream separator which had been previously heated with water at 180° F. and adjusted to recover plastic cream. The oil recovered from the cream spout was rewashed twice with hot water, to facilitate removal of any T-7 or serum solids remaining in it, and then reseparated. The yield of butteroil was 29 lb. or 92 per cent of the theoretical yield (31.5 lb.). Since the vanes of the separator had an appreciable amount of fat left on them, the actual efficiency on a larger scale operation undoubtedly would be greater.

Properties of T-7 butteroil. The refractive indices of butteroils made from the same cream, by de-emulsification with T-7 and by a conventional churning procedure, were compared and found to be identical ($n_D^{40} = 1.4547$). In order

to determine whether butteroil prepared by the T-7 method contained any residual agent, the active constituent being an alkyl sodium sulfate, sulfur analyses were obtained from an independent laboratory on two samples of butteroil from the same cream, one prepared by churning and the other with the aid of T-7. The samples were oxidized by the method of Niederl *et al.* (3) to convert all sulfur to sulfate and the sulfate sulfur determined as by Letonoff and Rheinhold (2). Sulfur contents of the churned and T-7 samples were 0.060 and 0.021 per cent, respectively. These data indicate that no appreciable amount of the alkyl sulfate remained in the oil. Both of the butteroils, when reemulsified in skimmilk had satisfactory flavor qualities.

DISCUSSION

The method of preparing butteroil by de-emulsification of cream appears to have certain advantages over known methods and its early publication, therefore, seemed justified. The method is economical and efficient. For plant operation a vat, equipped with an agitator, and a cream separator represents the necessary equipment. Incoming milk can be separated and the cream quickly converted into butteroil. The necessities of churning cream to butter or using specially designed equipment are eliminated and manipulation of the fat is kept to a minimum. When cream is supplied rather than prepared, a separator is not absolutely essential. The oil layer, obtained by de-emulsification, can be separated from the serum by siphoning, draining or decantation. Washing of the oil can be accomplished by similar procedures. The use of high-fat creams in the method seems specially recommended for two reasons. They require less agent for de-emulsification and they yield less serum, the usefulnss of which as a by product has not yet been established.

In all instances where incomplete de-emulsification was encountered during the investigation, the process could be completed by increasing the temperature and/or prolonging the holding time of the cream-agent mixture. Use of excessive amounts of T-7 should be avoided. High concentrations (in excess of 15 per cent) will reduce the yield of butteroil, even to the extent that in certain instances the creams are converted to heavy gels with no yield of oil.

Concerning the mode of action of the surface-active agents it may be significant to note that of 26 agents which de-emulsified cream quantitatively, 24 were of a cationic type, the other two being nonionic. A low molecular-weight solvent apparently is essential to effective action, since no pure agent as such was observed to perform satisfactorily in this study. Solution of the agent in such a solvent appears to facilitate dispersion in cream.

Where adequate precautions are taken, it seems unlikely that preparation of butteroil by the method reported would pose a toxicological problem. However, it seems advisable to reserve judgment on the usefulness of such butteroil until the purity and toxicology of the product have been investigated thoroughly. An extensive study of certain physical and chemical properties of butteroils prepared by several procedures is currently in progress at this laboratory.

SUMMARY

The possibility of preparing butteroil by de-emulsification of cream with surface-active agents was investigated.The potential utility for this purpose of 97 commercially available surface active agents was determined. Twenty-six of these demonstrated a capacity to quantitatively de-emulsify cream (40 to 45 per cent fat) at a level of 10 per cent or less of the agent. The testing procedure concerned addition of various amounts of agent to cream samples, heating the mixtures in a water bath, centrifuging the mixtures in graduated tubes and measuring the quantity of oil liberated. Most of the agents found effective yielded a clear quantitative oil layer before centrifuging. One of the effective agents (Tergitol 7) was studied intensively in regard to the variables of agent concentration, fat content of cream and heat treatment. Tergitol 7 was employed also in several pilot-scale productions of butteroil. Butteroil prepared with the aid of Tergitol 7 compared favorably in quality with butteroil produced from cream by churning. The de-emulsification procedure may have a number of useful applications in the dairy field and has certain significant advantages over present methods of manufacturing butteroil.

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BLOOD HEMOGLOBIN VALUES OF DAIRY CATTLE[1]

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The blood hemoglobin value of dairy cattle has been used as an index of the nutritional status of an animal with respect to iron, copper and cobalt. The literature is somewhat conflicting as to what constitutes a normal blood hemoglobin value for dairy cattle. Dukes (1) reports 12.2 g. hemoglobin per 100 ml. of blood as the normal hemoglobin value of the bovine species, whereas McCay (6) regards 10.9 g. as normal. Much of the conflicting evidence may be due in part to the many and varied methods of analysis. Many types of optical instruments have been used with variable results. Chemical analyses have not been too reliable. Inasmuch as difficulty is encountered in interpreting the literature with respect to a normal blood hemoglobin, additional data are desirable.

In connection with mineral studies of the Oregon Agricultural Experiment Station, the blood hemoglobin value has been used as an index of the iron, copper and cobalt status of dairy cattle throughout the state. This study reports the results of hemoglobin values obtained under various conditions.

EXPERIMENTAL

Holstein and Jersey cattle maintained by the Department of Dairy Husbandry of Oregon State College were available for obtaining blood samples at any time. Cooperating dairymen throughout the state submitted their cattle for the taking of blood samples upon request. Cattle from three distinctly different regions of Oregon were sampled. Information available does not suggest that the soil of any of these regions is mineral deficient.

Blood samples of 6 ml. were drawn from the jugular vein, using three drops of sodium citrate as an anticoagulant. Blood was hemolyzed by mixing with a quantity of saponin in a spot plate. Hemoglobin was determined by the use of the Spencer hemoglobinometer and recorded as grams of hemoglobin per 100 ml. of blood. Standardization of the Spencer hemoglobinometer was carried out by determination of total Fe on representative blood samples, using the method of Kennedy (3). While results with the Spencer hemoglobinometer and the Fe analysis were not in total agreement, they were relative and within the realm of practicability. Samples of blood from animals in the College herd were taken at regular intervals throughout the 2 yr. of the study. Other herds were sampled at infrequent intervals.

Although the feed of the animals varied, none of the animals was considered to be getting an abnormal ration. In many cases, the ration consisted entirely of home-grown feeds and, in others, purchased feeds and complex mineral supplements were fed.

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From preliminary results, a number of the animals in the College herd seemed to have a consistently low or a consistently high hemoglobin value. From these animals, groups of five low and five high Holsteins and five low and five high Jerseys were selected, blood samples being taken and hemoglobin values determined every 3 wk. for a period of 6 mo.

Since there is known to be a correlation between the age of the animal and some blood constituents, such as blood P (2), an analysis was made to determine any relationship between the blood hemoglobin values and the age of the animals. Preliminary observations suggested breed differences in blood hemoglobin values. Studies were planned to determine any difference in values of Holstein and Jersey cattle of both sexes.

RESULTS

A total of 1,014 blood samples representing 528 animals of the Jersey, Holstein, Guernsey, Brown Swiss and mixed breeds was analyzed. Represented were 14 Jersey males and 16 Holstein males with a total of 98 bleedings. Of the 498 females of all breeds sampled, there were 361 samples from 130 Holstein females, 461 samples from 274 Jersey females, and 94 samples of Guernsey, Brown Swiss and mixed breeds representing 94 individual animals. The results of the analyses are given in table 1.

TABLE 1

Average hemoglobin values by sex and breed

Breed	Sex	No. of animals	No. of samples	Av. hemoglobin values
				(*g./100 ml. blood*)
Jersey	Male	14	48	11.3
Holstein	Male	16	50	11.1
Jersey	Female	274	461	11.3
Holstein	Female	130	361	10.6
Mixed	Female	94	94	11.4
All males		30	98	11.2
All females		498	916	11.1
All Jerseys		288	509	11.3
All Holsteins		146	411	10.7
All animals		528	1,014	11.2

McCay (2) reports the blood hemoglobin values of males to be higher than females. On the basis of 70 samples on six mature bulls of four different breeds, McCay found that average blood hemoglobin value to be 12.8 g. per 100 ml. of blood, as compared to 10.9 $\pm$ 0.8 g. per 100 ml. for females. Table 1 shows that for Jerseys, 48 samples from 14 males averaged 11.3, whereas for Holsteins, 50 samples from 16 males averaged 11.1 g. of hemoglobin per 100 ml. of blood. The average of 98 samples from 30 Jersey and Holstein males is 11.2 g. of hemoglobin per 100 ml. of blood, considerably below the values reported by McCay.

The average hemoglobin value of 916 samples from 498 females was 11.1 g. Jersey males and females showed the same average of 11.3 g., whereas Holstein males averaged 11.1 g. and females 10.6 g., both lower than Jerseys and particularly the Holstein females.

Figure 1 represents the frequency distribution of the average hemoglobin value of 498 females sampled. The distribution is as uniform when only the Holstein or Jersey animals are considered as with all animals. The greatest number of animals (87.1 per cent) fall within the values of 9.0 and 13.0 g. of hemoglobin per 100 ml. of blood, and 45.5 per cent of the animals fall between the values of 10.5 and 12.0 g. per 100 ml. of blood. The variation in individual

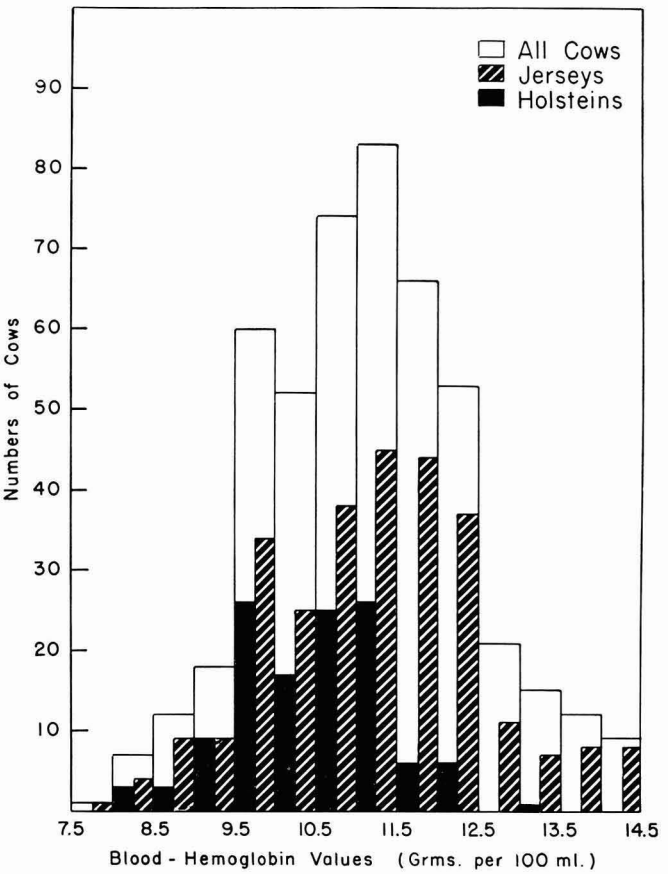

FIG. 1. Frequency distribution of blood hemoglobin values of 498 females.

animal values is indicated. When an animal shows low or high values, these most generally are always low or high. This is illustrated in table 2, showing the blood hemoglobin values of four animals all from the same herd, bled at various times over a period of 2 yr. Cows 230 and 494 maintained high hemoglobin values, and cows 225 and 456, low values during the period of observation.

Increases in hemoglobin values during the summer months are reported in the literature (3). To determine whether any differences could be shown between blood hemoglobin values during winter barn-feeding and summer pasture-feeding

conditions in Oregon, 20 cows were bled at approximately 3-wk. intervals. The results are shown in table 3. No consistent change occurred in the hemoglobin values of cows fed different rations in winter and summer.

Since fat is a major constituent of blood, the hemoglobin value of cows on a low-fat ration might vary from those on a high-fat ration. During the course

TABLE 2

Constancy of hemoglobin values

Breed	Animal no.	Dec. '48	Dec. '49	Mar. '50	Sept. '50	Av.
Jersey	230	12.5	13.7	12.8	13.0	13.0
Jersey	225	10.6	9.3	9.0	8.3	9.2
Holstein	494	12.0	11.5	11.8	11.5	11.7
Holstein	456	8.0	7.2	8.6	8.8	8.1

of the study, a group of 28 Holstein and Jersey cows was used on a feeding trial. Fourteen of the paired cows were fed a grain mixture containing 2.5 per cent fat, with the other 14 on a mixture containing 4.9 per cent fat. All cows were allowed free access to irrigated ladino clover and grass pastures. The blood hemoglobin values for the individual cows of the two groups are given in table 4. Analysis of variance shows no significant difference in the blood hemoglobin values of cows fed a low or high-fat grain mixture when on irrigated pastures.

TABLE 3

Blood hemoglobin values of the same cows during the winter and summer

Herd no.	Breed	No. of samples	Winter barn feeding	No. of samples	Summer pasture
456	Holstein	7	8.2	6	8.7
511	"	5	11.4	4	10.6
493	"	5	10.3	6	10.2
458	"	7	10.7	5	10.0
521	"	3	9.2	6	10.3
494	"	5	11.2	6	11.7
506	"	5	11.0	6	10.6
515	"	2	10.4	6	10.4
503	"	5	10.6	6	10.8
513	"	2	11.3	5	12.1
269	Jersey	6	9.8	5	10.0
200	"	5	11.4	6	11.8
212	"	6	10.2	6	10.2
271	"	4	10.4	6	9.0
225	"	7	9.8	6	10.4
220	"	7	11.9	6	12.3
219	"	6	10.9	6	12.2
210	"	4	12.6	6	12.9
230	"	5	12.8	6	12.1
226	"	2	12.0	5	11.9
Av.			10.8		10.9

Table 5 shows the average blood hemoglobin values of Holstein and Jersey females at 6-mo. age intervals. There appears to be no correlation of the age of the animal with the blood hemoglobin value. Peak values were reached at 2, 4.5 and 7 yr., and low values at 3.5, 5.5 and 7.5 yr. No significance could be attached to these variations.

TABLE 4

Blood hemoglobin values of cows on two levels of fat in the ration

Low fat			High fat		
Herd no.	No. of samples	Blood hemoglobin values	Herd no.	No. of samples	Blood hemoglobin values
		(*g./100 ml.*)			(*g./100 ml.*)
494	13	11.5	503	13	10.6
506	13	10.7	513	9	21.0
499	2	10.7	535	2	11.5
517	2	12.3	537	2	10.5
520	2	12.0			
533	2	9.5	516	2	12.4
536	2	10.3	456	13	8.5
522	2	11.6	534	2	10.4
523	2	11.3	500	2	10.9
215	2	11.4	200	12	11.7
			230	13	12.3
238	2	10.5	234	2	10.7
271	13	9.4	194	2	9.6
M-1	2	10.9	W-3	2	11.6
212	13	10.2	231	2	13.2
Av.		10.8			11.1

DISCUSSION

In the analyses to determine blood hemoglobin values, differences in the lots of saponin used to hemolyze the blood were encountered. Some of the newer lots of saponin did not hemolyze as rapidly or with as small amounts as did saponin that had been manufactured 15 to 20 yr. ago.

From 1,014 blood samples taken from 528 animals, the average blood hemoglobin value was 11.1 g. of hemoglobin per 100 ml. of blood. In the same herd under similar conditions, animals with low hemoglobin values apparently are

TABLE 5

Blood hemoglobin values as age increases

Age	No. of samples	Av. hemoglobin values
(*yr.*)		(*g./100 ml. blood*)
0.5	7	11.3
1	17	10.8
1.5	20	11.0
2	17	11.9
2.5	9	11.3
3	7	10.9
3.5	17	10.6
4	19	11.0
4.5	8	11.8
5	11	11.1
5.5	10	10.4
6	6	10.6
6.5	2	11.6
7	3	11.8
7.5	4	10.1
8	4	10.3
Over 8	6	10.7

quite common, as are animals with hemoglobin values higher than the average. Animals with low values generally continue to be low, and animals which were high in the early bleedings continued to show a high blood hemoglobin value throughout the study, regardless of the ration fed.

No significant difference between hemoglobin values of Jersey and Holstein males and Jersey and Holstein females has been shown in this study. The average blood hemoglobin value of the males was 11.2 g. per 100 ml. of blood, which is considerably below McCay's (6) value of 12.8 g. per 100 ml. of blood. In this study, the 30 males were all under 2 yr. of age, whereas McCay's results are based on six mature bulls.

We have not been able to confirm the results of Kroncher (4, 5) and Van Geller (7), who have shown that the hemoglobin value of cows increased during the summer on pasture. Our results support those of McCay (6), who showed no significant difference between the blood hemoglobin values during barn and pasture feeding.

The results in table 4 indicate that the hemoglobin values of cows on a high-fat ration tend to be higher than those on a low-fat ration, even though the difference is not statistically significant.

The blood phosphorus values of dairy cattle show a distinct correlation with the age of the animal (2). In this study, no statistically significant differences could be found in the hemoglobin values of cows at different ages. The Holstein breed shows a trend towards lower values at advanced ages, but this may be due in part to sampling. At the advanced ages, a small number of animals are represented even though many samples on the individual animals were obtained.

The literature reports conflicting evidence as to a breed difference in blood hemoglobin values. In this study, a statistically significant difference between the Holstein and Jersey breeds was found. The Jerseys were found to have an average blood hemoglobin value of 11.3 g. and the Holsteins, 10.6 g. per 100 ml. of blood. McCay (6), in his work, showed no relation between blood hemoglobin values and breed.

CONCLUSIONS

The average blood hemoglobin value of Holstein, Jersey, Guernsey and grade cattle in this study was 11.1 g. of hemoglobin per 100 ml. of blood.

The average blood hemoglobin of males did not differ significantly from that of females.

Animals with low or high hemoglobin values generally maintain low or high values over extended periods.

Pasture feeding did not change the blood hemoglobin value significantly from barn feeding on a group of 20 cows bled over a period of 1 yr.

A high-fat ration gave a blood hemoglobin value higher than a low-fat ration, although the difference was not statistically significant.

There was no significant correlation of the blood hemoglobin of dairy cattle and age.

There is a statistically significant difference between the blood hemoglobin

value of Holstein and Jersey cattle. The average Holstein blood hemoglobin value was 10.6 g., whereas the Jersey breed was 11.3 g. per 100 ml. of blood.

Since the blood hemoglobin value of normal dairy cattle varies widely, great caution should be exercised in the interpretation of the data.

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MULTIPLE STRAIN BACTERIOPHAGE INFECTIONS OF COMMERCIAL LACTIC STARTERS[1]

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In testing for the presence of bacteriophage in multiple-strain starters and cheese whey from plants experiencing delayed acid development during the cheese making process, whey filtrates were obtained with such broad patterns of activity that the presence of several strains of bacteriophage was suspected. The work reported here was carried out to determine the number and some of the characteristics of individual bacteriophage strains that might be found in such samples. A preliminary report was published in abstract form (1).

EXPERIMENTAL METHODS

The starter cultures and whey samples were obtained from commercial cheese plants. Bacteria-free whey filtrates were prepared by filtering the samples first through sterile, coarse filter paper and then through a Selas porcelain filter (porosity #03). These whey filtrates were stored in screw-cap bottles at 2 to 3° C. Care was taken to avoid laboratory contamination with other bacteriophages.

Presence of bacteriophage in the whey filtrates was determined by inoculating 7 ml. of sterile litmus milk, enriched with 1 per cent non-fat dry milk solids and 10 per cent V-8 juice, with one drop each of an 18- to 20-hr. culture of the lactic streptococcus tested and of the whey filtrate. Controls, from which the filtrate was omitted, also were prepared. The presence of bacteriophage active against the organism used was indicated by the lack of normal acid development and reduction following incubation for 18 to 20 hr. at 32° C. The results of these tests were very consistent. The lactic streptococci used in these studies were from the laboratory stock collection or obtained as fresh isolates from multiple-strain commercial cultures and were selected to cover a wide sensitivity range.

The bacteriophage strains present in two of the whey filtrates were isolated from individual plaques on plaque plates. Trypticase soy agar medium, to which 0.02 per cent L-cystine had been added, was used and the two-layer plating technic, with 0.33 per cent $CaCl_2$ in the overlay, as reported by Potter and Nelson (3), was employed. Single plaques were picked into tubes of sterile skimmilk, one drop of the host organism culture added and the tubes then held at 32° C. for 8 hr. Bottles containing 100 ml. of sterile skimmilk then were inoculated with 1 per cent of the material from the test tube propagation and 1 per cent of the same host culture. After incubation for 15 to 16 hr. at 32° C., 10 per cent lactic acid was added to coagulate the casein and a cell-free filtrate was prepared in the same manner as employed for the original filtrate.

The filtrates thus prepared from whey filtrate F83 were carried through two

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additional plaque isolations to be more certain that the final filtrates contained a single strain of bacteriophage. Preparations of these more highly purified strains of bacteriophage were examined by means of an R.C.A. type EMU electron microscope, using a procedure of Parmelee *et al.* (2).

RESULTS AND DISCUSSION

Information concerning the sources of the samples examined in this study is presented in table 1. Cultures B1, NL and AT originally were obtained from this

TABLE 1

Sources of starter cultures and cheese whey samples

Date (1951)	Plant	Material examined	Original culture	Phage activity	Filtrate no.
Feb.	A	Bulk culture	Unknown	No	.....
		Blue cheese whey	Unknown	Yes	F83
Feb.	B	Blue cheese whey	B1	Yes	F84
Feb.	C	Mother culture	B1	Yes	F85
		Buttermilk	B1	Yes	B1S
		Bulk whole milk culture	B1	Yes	B1W
		Mother culture	NH	Yes	F86
		Bulk culture	NL	Yes	F87
March	D	Cottage cheese whey	B1	Yes	F88
April	B	Cottage cheese whey	AT	Yes	F89

laboratory and have been used in a number of commercial plants. Culture NH was procured by plant C from a commercial laboratory. The identity of the culture used in Plant A was not known but it had been used for about 1 yr.

Only one of the cell-free filtrates, that from the bulk culture from plant A, failed to show bacteriophage activity against some of the test organisms. The activity patterns of the unpurified active filtrates on 13 test organisms are shown in table 2. These test organisms were selected as representing those most sensi-

TABLE 2

Activity patterns of bacteria-free whey filtrates from original samples

Test org.	Bacteriophage activity[a] of filtrate:								
	F83	F84	F85	F86	F87	F88	F89	B1S	B1W
H1–2	+	+	+	+	+	+	+	+	+
H1–4	+	−	−	−	−	+	−	−	?
5	+	−	−	−	+	−	−	−	+
KH	+	−	−	+	−	−	+	−	+
799	+	−	−	?	+	−	−	+	−
122–2	+	+	+	+	+	+	−	+	+
DL	+	−	−	−	−	−	−	−	−
HC-2	+	−	−	−	+	+	+	−	−
FD–56	−	−	−	+	+	−	−	+	−
B1–5	−	+	−	−	?	−	−	+	+
B1–6	+	+	+	+	+	−	+	+	+
B1–24	−	−	−	−	−	−	+	−	−
B1–28	+	+	+	+	+	−	+	+	+
Plant	A	B	C	C	C	D	B	C	C

[a] Bacteriophage activity: + = positive, − = negative, ? = questionable.

tive to these bacteriophages, a large number of organisms not being included in the table because they gave negative results. The B1 cultures were unidentified lactic streptococci isolated from the multiple-strain culture B1 carried in this laboratory. The others were from the stock collection and the majority had been identified as *Streptococcus cremoris*. While some of the test cultures listed were sensitive to all or a majority of the unpurified filtrates, others were quite resistant. The five different filtrates from plant C showed a number of common activities against test cultures, but several of these filtrates showed activities not possessed by others, even though three of the filtrates were from material inoculated with commercial culture from the same original source. The same lack of complete agreement existed with the two filtrates from plant B, when two different starter cultures were involved.

Filtrates F84 from plant B and F85 from plant C had very similar activity patterns. Both of these filtrates came from material inoculated with culture B1 within a few days of the same time. The activity patterns of the other filtrates, however, were quite different. This was true of the filtrates from different plants using the same original starter, as well as those prepared from different products of the same plant set with the same strain of mother culture during an interval varying from a few days to a week. The conclusion may be drawn then that the bacteriophage activity range of a whey filtrate from a phage-contaminated culture cannot be predicted, even though the sensitivity patterns of other whey filtrates from material inoculated with the same original culture but infected at a different plant or different time are known.

The activity pattern of the bacteriophage strains isolated from filtrate F83 and purified by three successive single-plaque isolations are presented in table 3. The plaque counts given represent the number of bacteriophage particles of each strain demonstrated in the original whey filtrate. Strains 3, 4 and 5 were isolated following propagation of the original whey filtrate, rather than from the filtrate itself, and no original plaque count data were obtained. Strains 3 and 5 were propagated with organism KH and strain 4 with organism 5 before they were isolated; strain 3 subsequently was purified and propagated on organism H1–4.

The data presented in table 3 show that phage strains 1 and 5 gave the same reactions with the test organisms used; these two strains probably are essentially identical. Of the other nine strains isolated, four lysed only the host organism employed for isolation, while the other five lysed at least one additional test organism.

The plaque counts of the strains isolated directly from the whey filtrate F83 ranged from 1×10^4 to 900×10^4 phage particles per milliliter. These plaque counts were made at different intervals, as long as 1 mo. with some strains, after the original whey filtrate was prepared. Higher plaque counts might have been obtained if all strains had been isolated when the filtrate was fresh.

Considerable variation in the diameters of plaques produced by the organism-bacteriophage combinations was observed even after three-fold purification of the bacteriophage strains. This variation was so pronounced that the value of a

plaque diameter measurement as a strain characteristic would be limited. There was some evidence that the strain of host bacteria used influenced the plaque size. For example, a bacteriophage strain capable of lysing the test organism H1–2 produced larger plaques with this organism as host than with any of the other susceptible test organisms used in this study.

As a further demonstration of the specificity of certain of these isolated strains, bacteriophage strain 6 was shown not to produce plaques with B1–5 as the host organism, as did strain 9, indicating that the common ability to lyse 122–2 was not related to the ability to lyse B1–5. Phage strain 11 failed to lyse organism

TABLE 3

Activity patterns of purified bacteriophage strains isolated from filtrates no. F83

Test org.	Orig. filtrate F83	Bacteriophage activity of purified strain no.										
		1	2	3	4	5	6	7	8	9	10	11
H1–2	+[a]	+[b]	–	–	–	+	–	–	–	–	–	–
H1–4	+	–	+[b]	+[b]	–	–	–	–	–	–	–	–
5	+	–	–	–	+[b]	–	–	–	–	–	–	–
KH	+	+	–	+	–	+[b]	–	–	–	–	–	–
799	+	–	–	–	–	–	–	–	–	–	–	–
122–2	+	–	–	–	–	–	+[b]	–	–	+	–	–
DL	+	–	–	–	–	–	–	+[b]	–	–	–	–
HC–2	+	–	–	–	–	–	–	–	+[b]	–	–	–
FD–56	–	–	–	–	–	–	–	–	–	–	–	–
B1–5	–	–	–	–	–	–	–	–	–	+[b]	–	–
B1–6	+	–	–	–	–	–	–	–	–	–	+[b]	+
B1–24	–	–	–	–	–	–	–	–	+	–	–	–
B1–28	+	–	–	–	–	–	–	–	–	–	+	+[b]
A15–2	[c]	–	–	–	–	–	–	–	–	–	+	–
Date isolated	2/21	3/8	3/8	2/22	2/22	2/22	3/1	3/9	3/8	3/20	3/13	3/20
Plaque count/ml. F83 ($\times 10^4$)		1	27.5	[d]	[d]	[d]	120	400	16	2	900	108
Range in plaque size diam. (*mm.*)		2.0 to 4.5	1.0 to 0.15	0.5 to 1.5	0.25 to 0.75	0.75 to 1.6	0.50 to 2.0	0.50 to 1.25	0.75 to 2.50	0.60 to 1.50	0.50 to 2.0	0.50 to 1.50

[a] Bacteriophage activity: + = positive, – = negative, ? = questionable.
[b] Host organism for plaque isolation and purification.
[c] Organism isolated too late to determine sensitivity to original whey filtrate F83.
[d] Plaque count not made on original whey.

A15–2 in litmus milk, but it did lyse this organism and B1–6 in addition to its host B1–28 in plaque plate preparations, thus having the same activity pattern as strain 10 displayed. Since the other characteristics of strains 10 and 11 were almost identical, the failure of strain 11 to lyse organism A15–2 in litmus milk was not considered an adequate basis for recognizing these two bacteriophage strains as being very different. Propagation upon two different host organisms might explain the slight difference in activity shown by the two strains.

The morphology of the purified strains from F83, as observed with an electron microscope, indicated no differences between strains. The morphological charac-

teristics and measurements were the same as previously reported for lactic streptococcus bacteriophage by Parmelee *et al.* (2).

Whey filtrate F89 also was studied further because of its activity against several of the test cultures, including B1–24, a culture that had been quite resistant to filtrates previously studied. The titer of the isolated strains present in the original whey, their activity patterns and range in plaque size are presented in table 4.

TABLE 4

Activity patterns of bacteriophage strains after initial isolation from filtrate F89

Test org.	Original filtrate	Bacteriophage activity[a] of isolated strain no.						
		1a	2a	3a	4a	5a	6a	7a
H1–2	+	+[b]	−	−	−	−	−	−
H1–4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
KH	+	+	+[b]	−	−	−	−	−
799	−	−	−	−	−	−	−	−
122–2	−	−	−	−	−	−	−	−
DL	−	−	−	−	−	−	−	−
HC–2	+	−	−	+[b]	−	+	−	−
FD–56	−	−	−	−	−	−	−	−
B1–5	−	−	−	−	−	−	−	−
B1–6	+	−	−	−	+[b]	−	+	+
B1-24	+	−	−	−	−	+[b]	−	−
B1–28	+	−	−	−	+	−	+[b]	+
A15–2	+	−	−	−	+	−	−	+[b]
Date isolated	4/27	4/30	4/30	4/30	4/30	4/30	4/30	4/30
Plaque count/ml. F89 ($\times 10^4$)		500	24,000	800	200	0.03	200	315,000
Range in plaque size diam. (*mm.*)		2.5 to 4.5	2.0 to 2.5	7.5 to 1.5	7.5 to 2.0	1.0 to 1.5	0.75 to 2.0	0.3 to 1.75

[a] Bacteriophage activity: + = positive, − = negative.
[b] Host organism for plaque isolation.

Of the seven strains isolated, six were found to have different sensitivity patterns. Strains 4a and 7a lysed the same test cultures, although their plaque counts from the original whey were quite different, as were the plaque-size ranges.

Comparison of activity patterns of the strains isolated from filtrate F83 with those from F89 shows that the activity of strains isolated and propagated on the same host organism is not always the same. Strain 5, isolated from F83 with KH as the host organism, lysed organism H1–2. Strain 2a, isolated from F89 with KH as the host, did not lyse H1–2 in either litmus milk or in agar plate preparations. Strain 8 from F83 lysed B1–24 in addition to its host organism HC–2, while strain 3a isolated from F89 with the same host organism failed to lyse B1–24. It also was found that, while the bacteriophage strain isolated from F83 with organism B1–28 as the host would lyse organism A15–2 in a plaque plate preparation, the strain from F89 isolated with B1–28 would not lyse A15–2. On the other hand, a number of the strains obtained from F89 had activity patterns the same as those of strains from F83.

Isolation of these several different strains of bacteriophage from filtrates F83 and F89 indicates that the original starters used in these plants undoubtedly were multiple-strain cultures, a fact corroborated by actual strain isolations made from several of the mixed cultures.

No attempt was made to isolate the individual bacteriophage strains from the remaining whey filtrates, although from the data presented in table 2 it is evident that a number of strains probably was present in each.

Since some bacteria-free filtrates obtained from cultured dairy products prepared with different commercial starter cultures would lyse the same organisms, it seems evident that these commercial cultures contained one or more closely related, if not identical, strains of lactic streptococci. The common practice of laboratories preparing lactic cultures has been to combine several different strains of lactic streptococci in an effort to obtain a starter that will function properly when exposed to a variety of conditions. In a reasonably well balanced multiple-strain culture, slowness may not become an important factor unless a large per cent of the organisms present are susceptible to the bacteriophage strains that gain entrance to the culture. Quite probably the presence of bacteriophage active against but one organism strain would not be apparent in many mixed cultures under plant conditions. When a plant using one of the common commercial cultures does experience culture slowness due to bacteriophage, it is probable that either more than one organism strain has been lysed, due to a multiple-strain bacteriophage infection, or that the culture consists mostly of one strain that has outgrown the others and now has been lysed.

Probably all strains of bacteriophage found in a multiple-strain infection do not have a common origin; undoubtedly they may enter at different points in the preparation and handling of the culture material. This is pointed out by the results recently obtained when cell-free filtrates prepared from a mother culture, the bulk culture and the vat of "slow" cheese milk inoculated with the bulk culture, were tested for the presence of bacteriophage. While none of the organisms isolated from the mother culture was affected by the filtrate from the mother culture, many were lysed by both the filtrate from the bulk culture and that from the cheese milk. The mother culture then was propagated in the presence of the filtrate from the bulk starter and the sensitivities of 19 isolates from this culture tested. None was lysed by the bulk culture filtrate but two were lysed by the cheese milk filtrate, indicating the presence of at least one additional bacteriophage strain in this latter filtrate. The filtrate prepared from the mother culture was not entirely free of bacteriophage, since it definitely lysed the test organisms FD–56 and A15–10 and gave questionable results with some others when tested in litmus milk. The filtrates prepared from the bulk starter and cheese milk definitely lysed FD56 and eight other test organisms of the 14 listed in table 3 and 4, although A15–10 was not lysed. Unfortunately the intermediate culture, inoculated from the mother culture and subsequently used to inoculate the bulk culture, was not available for testing and it was not possible to determine if the increase in bacteriophage infection was initiated with the intermediate culture or the bulk culture prepared from it.

As long as a dairy plant is contaminated with bacteriophage, the succeeding starter culture may not be satisfactory for any length of time, with the usual methods of handling, unless its component strains are not affected by the bacteriophage strains present. Such resistant cultures may be difficult to obtain, since the identity of the component strains usually is not known. It also is possible that one or more strains present in the replacement starter would be identical with or closely related to those strains in a previous starter that had been attacked by the bacteriophage.

The possibility exists that had other test cultures been employed in the present studies a greater number of bacteriophage strains might have been demonstrated in some of the samples. Maintenance of an adequate collection of test cultures becomes a definite problem in screening for the presence of bacteriophage in samples from a variety of sources. The question as to whether the manipulations employed in the isolation and propagation of the various bacteriophage strains influenced the range of activity to a significant degree is unanswered, although this may be a problem of major importance in control of bacteriophage active against lactic streptococci.

SUMMARY

All but one of ten samples of whey or culture from four dairy plants experiencing "slowness" in their lactic cultures or products contained lactic streptococcus bacteriophage. The activity pattern of the bacteria-free whey filtate prepared from each of these nine samples indicated a multiple-strain bacteriophage infection.

On the basis of the test cultures lysed, ten different bacteriophage strains were isolated from a blue cheese whey and six (possibly seven) from a cottage cheese whey. Electron micrographs of the ten bacteriophage strains isolated from the blue cheese whey revealed that all were similar in morphology.

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THE VALUE OF VITAMIN B_{12}, DL-METHIONINE AND POTASSIUM-PENICILLIN IN MILK REPLACEMENT FORMULAS FOR DAIRY CALVES[1, 2]

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The development of a milk replacement formula (6, 8) and the widespread use of it by dairymen has shown that calves can be raised successfully with small amounts of saleable whole milk. Significantly increased growth rates in calves, due to ration supplementation with an APF supplement have been reported (2), while other workers (4, 7) have not been able to demonstrate such increases.

The research presented in this report was undertaken in part to furnish additional data on the role of vitamin B_{12} in calf nutrition. Increased growth rates reported in poultry rations supplemented with 0.3 per cent DL-methionine (1, 3) prompted its use, at the same level, in this work. The use of potassium-penicillin as a milk replacement supplement also was investigated in this trial.

EXPERIMENTAL PROCEDURE

The male Holstein calves used were obtained during the winter of 1951 from Pennsylvania state institutional herds. They were housed in individual solid-walled pens equipped with a water bowl, a salt block and a feeding box for the calf starter. To prevent positional effects, the calves were placed at random throughout the artificially lighted and ventilated stable, maintained at a temperature of 65° F. by steam heat controlled thermostatically. Three measures of growth (body weight, height at withers and chest circumference) were taken each week by the same person and at the same time of day. Daily observations were made of the condition of the feces of each calf. If scours persisted for 24 hr., an 8-g. dose of sulfathalidine was administered orally, followed by an additional 4-g. dose at each of the next two successive feedings.

Twenty-four calves were divided into four groups of six calves each, which were comparable on the basis of body weight, chest circumference and height at withers. All calves were placed on experiment before they were 5 days old.

Group I (control) was fed the following milk replacement formula: 50 lb. dried skimmilk, 10 lb. dried whey, 15 lb. distillers' dried corn solubles, 10 lb. soluble blood flour, 7 lb. dextrose, 5 lb. oat flour, 0.5 lb. vitamin A and D concentrate (4000 U.S.P. units and 500 U.S.P. units per gram) 0.5 lb. trace elements and 2 lb. dicalcium phosphate. Throughout the milk replacement feeding period the control replacement was supplemented as follows: Group II received 50 g. vitamin B_{12} supplement (6 mg. vitamin B_{12} per pound); group III received

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0.3 per cent DL-methionine; and group IV received 0.5 g. potassium-penicillin per 100 lb. milk replacement.

The milk replacements were dissolved in water at 100° F. and were fed from open pails placed in the concentrate box located 16 in. above the floor of the pen. The rate of feeding was: First through 4th day—dam's milk; 5th through 7th day—2 lb. whole milk, 0.2 lb. milk replacement, 2 lb. water (twice daily); 8th through 10th day—1 lb. milk, 0.4 lb. milk replacement, 3 lb. water (twice daily); 11th through 21st day—0.5 lb. milk replacement and 5 lb. water (twice daily); 22nd through 35th day—0.6 lb. milk replacement and 6 lb. water (twice daily); 36th through 49th day—0.7 lb. milk replacement, 6 lb. water (twice daily); and 50th through 56th day—0.7 lb. milk replacement, 7 lb. water (once daily).

All groups of calves were fed *ad libitum* a good quality timothy-alfalfa hay during the trial. Calf starter was fed *ad libitum* until each calf, if possible, was able to consume the maximum of 6 lb. daily for the duration of the 12-wk. trial. The calf starter was prepared as follows: 416.5 lb. ground yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (44 per cent protein), 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skimmilk, 100 lb. distillers' dried corn solubles, 0.5 lb. irradiated yeast (4,000,000 U.S.P. units vitamin D per pound in dry meal form), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt, and 3 lb. vitamin A feed (4,000,000 U.S.P. units per lb.).

EXPERIMENTAL RESULTS

The growth data for these trials are summarized in table 1. When all the growth data were treated statistically (5), no significant differences in growth

TABLE 1

Summary of growth data[a]

Group	No. of calves	Body weight		Withers height		Chest cir.	
		9 wk.	12 wk.	9 wk.	12 wk.	9 wk.	12 wk.
		(*lb.*)		(*in.*)		(*in.*)	
I (Control)	6	0.94	1.17	0.05	0.05	0.07	0.09
II (Vit. B_{12})	6	0.84	1.12	0.04	0.05	0.06	0.07
III (DL-methionine)	6	0.89	1.23	0.05	0.05	0.07	0.09
IV (Penicillin)	6	0.40		0.03		0.04	
	4		0.81		0.04		0.06

[a] Expressed as mean daily gains.

rates were found at 9 and 12 wk. between the control group and the DL-methionine and vitamin B_{12}-supplemented groups. These negative findings with vitamin B_{12} supplementations of milk replacements corroborate previous results obtained (7).

The supplementation of a milk replacement with potassium-penicillin resulted in a depression of growth rates. The mean daily gains of the control calves at 9 and 12 wk., respectively, were 0.94 and 1.17 lb. per day, while those for the penicillin-supplemented calves were 0.40 and 0.80 lb. per day. The differences between these rates of gain in weight were significant at the 1 per cent

level at 9 wk. in a complete group analysis and significant at the 5 per cent level at 12 wk. in an analysis necessitating the use of estimated values. Estimated values were introduced into the data when two calves in the penicillin-supplemented group died of pneumonia at 9 and 10 wk. of age, respectively, at the time when the replacement feeding period had been terminated and subsistence on starter and hay had become necessary. It also was noted that the penicillin-supplemented group had a greater incidence of respiratory ills, as compared with the other calves on this trial.

All calves drank the milk replacement mixtures readily, but there was a large

TABLE 2

Summary of calf starter consumption (lb.)

Group	Total starter for 6 calves	1st 8 wk.	Starter consumed per calf		
			9th & 10th wk.	11th & 12th wk.	Lb. starter/ lb. gain (12 wk.)
I (Control)	1268.0	68.0	63.0	80.0	2.24
II (Vit. B_{12})	1128.0	48.0	57.6	81.5	2.04
III (DL-methionine)	1295.5	56.7	65.9	93.0	2.07
IV (Penicillin)	613.0[a]	24.5	30.0	70.0[b]	2.02

[a] Total for 6 calves.
[b] Based on 4 surviving calves.

difference in the amount of starter consumed, as shown in table 2. The average starter consumption per calf for the control group was 211.3 lb., while that for the penicillin-supplemented group was 102.2 lb. Hence penicillin-fed calves consumed about one-half the amount of starter as did the control calves during the first 10 wk. of the trial. During the last 2 wk. of the trial, the four surviving calves, which had been fed a penicillin-supplemented milk replacement, ate considerably more starter, approaching that amount consumed by the controls over the same period. However, hay consumption among the penicillin-fed calves was not depressed.

SUMMARY

The addition of vitamin B_{12} as well as DL-methionine to a milk replacement formula did not increase growth rates of young dairy calves. These negative findings with vitamin B_{12} supplementation corroborate former work done at this station. A milk replacement containing potassium-penicillin significantly decreased the rate of gain in weight of dairy calves and lowered the amount of starter consumed by them.

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FACTORS UNRELATED TO VITAMIN A INTAKE THAT INFLUENCE PLASMA VITAMIN A CONCENTRATION

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A number of investigators have studied the relationship of the level of vitamin A in blood plasma to the intake and storage of this nutrient in animals (2, 3, 5, 6, 7, 9, 10, 11). In general, the levels in the plasma reflected differences in levels of intake and storage. However, there is a limited amount of data showing irregularities or even an inverse relationship between blood plasma levels and intake or storage (4, 7, 8, 9, 14). This relationship needs to be investigated further because of the importance and general use of blood plasma levels to estimate adequacy of the diet. The observations and experiments described in this and in another report (15) point out some of the limitations which apply when blood plasma levels of vitamin A are compared or related to level of intake of carotene and/or vitamin A. They also show some inconsistencies with the generally accepted relationship.

EXPERIMENTAL PROCEDURE

Two experimental procedures were employed to show irregular changes in plasma vitamin A levels caused by diet changes. In the first experiment, 39 calves were reared from birth in a normal manner; they were fed limited amounts of whole milk until 60 days of age and grain and alfalfa until 90 days of age. Twenty-one of the calves were fed 25,000 or 50,000 I.U. of vitamin A daily as cod-liver oil for 27 to 90 days, and 18 received no vitamin A supplement. At 90 days of age all calves were placed on a carotene-vitamin A-free diet consisting of grain and skimmilk (7). Wood shavings were used as bedding.

In the second experiment, calves ranging in age from 2 to 197 days were subjected to various dietary changes to determine the relationship of these changes to plasma vitamin A levels. The calves were reared on grain and milk, although a few also received 0.5 to 1 lb. of alfalfa hay per day. While on grain and whole milk, each calf was given daily a capsule containing 140,000 I.U. of vitamin A for a period of 3 to 7 days. One wk. or more after the last capsule was given and when plasma vitamin A levels had been found to be fairly constant the diet change under study was made. Most diet changes ran for about 1 to 2 wk., and several successive diet changes were made on the same calf during the first few months of its life. The dietary changes consisted of a change (a) from whole milk to skimmilk and then back to whole milk, (b) from whole to skimmilk plus lard and then to skimmilk, (c) from whole milk to skimmilk plus choline and (d), in one case, from a semi-synthetic ration to the same diet minus fat and vitamin A.

The rations employed maintained plasma carotenoid levels at a minimum. Thus, in the modified Kimble method used for the determination of plasma vita-

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min A, the correction for carotoids in the Carr-Price reaction always was very small and was not a source of error or variation.

RESULTS AND DISCUSSION

Experiment 1. Examples of the changes in plasma vitamin A levels observed when the calves were changed from whole to skimmilk are shown in figure 1. Calf 2725 had received 50,000 I.U. of vitamin A per day until 70 days of age and at 90 days of age the diet was changed from alfalfa and grain to grain and skimmilk. After this diet change, a pronounced increase in plasma vitamin A level was found for the next 30 days, followed by a gradual decrease. Another calf, 2718, had received only 25,000 I.U. of vitamin A for 31 days and when the diet change was made at 90 days of age only a small increase in plasma vitamin A level was noticed during the second week. The values for calf 715, which received no vitamin

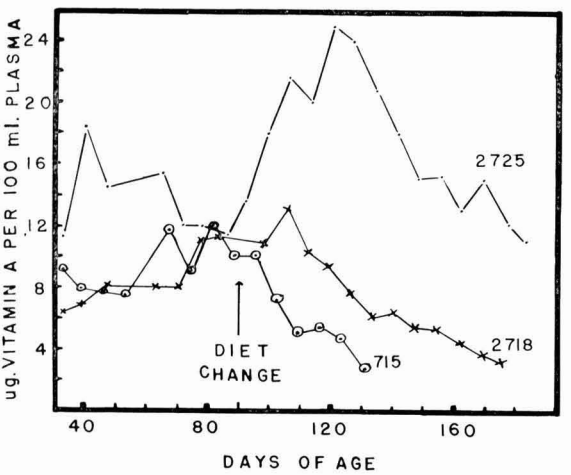

FIG. 1. Changes in plasma vitamin A concentration of 3 calves when all were placed on a deficient diet at 90 d. of age.

A supplement, showed the generally expected decrease when the calf was placed on the deficient diet consisting of skimmilk and grain. These three examples were typical of others on the experiment and illustrate the unexpected increase that frequently was observed when calves with a large storage of vitamin A were changed from a ration with adequate carotene and vitamin A to a skimmilk ration which was deficient in carotene and vitamin A.

In the first experiment, when the 18 normally fed calves that had received no supplemental vitamin A were changed to grain and skimmilk, 13 showed a gradual decrease in level of vitamin A in blood plasma, as illustrated by calf 715 in figure 1. Five showed an increase of plasma vitamin A for only 1 wk. after the change. The five values averaged 3.9 γ per 100 ml. of plasma above the level before the diet was changed.

In the group of 21 calves that received supplemental vitamin A, 13 showed a definite increase in plasma vitamin A level when the same ration change was

made. The increase averaged 3.7 γ per 100 ml. plasma and ranged from 1 to 10 γ above the level before the diet was changed. The increase persisted for 1 to 7 wk. after the calves were changed to the deficient diet. The majority of the calves that showed this "unexpected" increase had received the largest amounts of supplemental vitamin A when on the normal diet.

Experiment 2. An example showing the sequence of diet changes and their effect on the level of vitamin A and carotenoids in the plasma of one calf is presented in figure 2. An increase in vitamin A concentration was observed when skimmilk replaced whole milk when the calf was 49 days of age and again at 71 days. A rapid decrease was observed when whole milk replaced skimmilk at 78

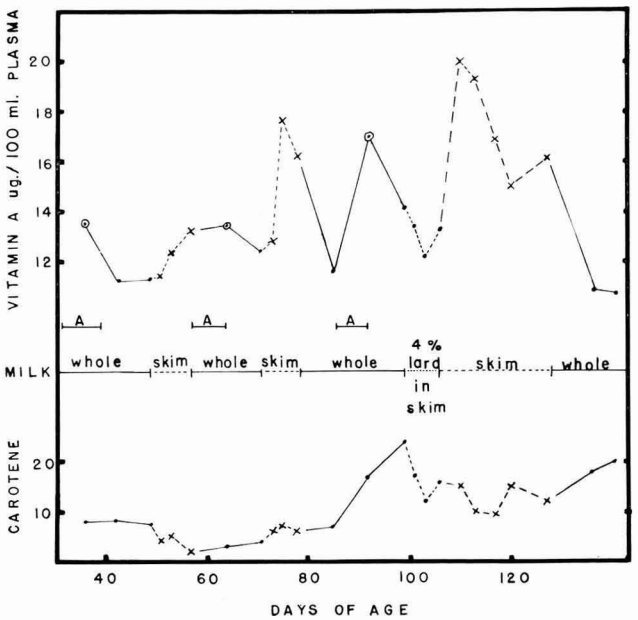

FIG. 2. The effect of a sequence of diet changes in the milk portion of a diet of grain and milk on the plasma vitamin A level of calf no. 2963.

and 127 days. It is evident that values after the third day on the skimmilk diet, which was deficient in vitamin A, were frequently higher than when receiving whole milk and a vitamin A supplement of 140,000 I.U. per day. During this experiment it became apparent that a larger percentage of the younger calves responded with an increase in plasma vitamin A levels than in the case of the older calves when skimmilk replaced whole milk. A summary of data showing the effect of this diet change on plasma vitamin A levels is presented in table 1.

The calves have been divided into two groups, those less than 90 days of age and those which were more than 90 days of age when the change was made. In the former there were 16 calves with 37 changes, and in 35 of these changes a pronounced increase in plasma vitamin A level was observed when skimmilk was

substituted for whole milk. In the ten older calves, 12 out of 16 changes showed this increase. The unexpected increase was highly significant by the t test (13) for the 3- to 8-day period after the ration change, when it was compared to the values observed at the time of the ration change in the younger calves. In the older calves the same trend was shown, but individual variation in absolute levels and in degree of response was so large that the differences were not statistically significant.

The plasma vitamin A values showed an immediate and pronounced decrease from 14.5 to 10.5 γ per 100 ml. in 19 cases in the younger calves when whole milk replaced the skimmilk, which had been fed for only 7 to 10 days.

TABLE 1

Average plasma vitamin A values of calves when changed from whole milk to skimmilk

	During vitamin A supplementation	1 wk. later on day of diet change	Days on skimmilk				± 7 d. after diet changed back to whole milk
			1st–2nd	3rd–4th	5th–8th	9th–14th	
Calves under 90 d. of age (37 changes involving 16 calves)							
No. of determinations	30	37	35	36	35	12	19
Plasma vitamin A (γ/*100 ml.*)	14.8	10.6	11.6	13.1	14.0	11.9	10.5
Standard deviation	±4.3		±3.0	±3.2	±3.4	±2.6	±4.7
Significance by t	**[b]		n.s.[a]	**	**	n.s.	n.s.
Calves over 90 d. of age (16 changes involving 10 calves)							
No. of determinations	7	16	13	15	16	7	0
Plasma vitamin A (γ/*100 ml.*)	14.6	12.5	12.8	13.9	13.6	13.0	.....
Standard deviation	±3.8	±3.4	±3.4	±4.2	±4.6	±4.0	.....
Significance	n.s.	.....	n.s.	n.s.	n.s.	n.s.	.....

[a] n.s. = not significant (at P = 0.5) from value on day of diet change.
[b] ** = highly significant (P = 0.01).

Since the only diet change in experiment 2 was the milk, a possible explanation for the greater incidence of this unexpected increase in the younger calves might be that the larger proportion of their nutrients came from milk, whereas in the older calves the larger proportion of their nutrients came from grains. Therefore, some component of milk may have been the factor causing the unexpected increase.

In order to study the effect of another fat, eight trials were performed on eight different calves in which whole milk was replaced by skimmilk to which was added 4 per cent of commercial lard. The mixture was homogenized at 3,000 lb. per in.^{2} before feeding. After this dietary change, a small decrease in plasma vitamin A level was observed. This decrease could be ascribed to decreased intake, and it was not statistically significant (table 2).

After the skimmilk-lard mixture was fed for 7 days, skimmilk was substituted for the skimmilk-lard mixture in seven of the trials with seven calves. The plasma vitamin A level increased as a result of this diet change, just as it did when skimmilk replaced whole milk. The average values for these dietary

changes are given in table 2 and illustrated in figure 2 for calf 2963 at 106 days of age.

In addition, one calf was changed from a semi-synthetic milk containing casein, corn sugar, lactose, lard, vitamins (including vitamin A) and minerals to the same diet containing no lard or vitamin A. The plasma level increased from 5.8 to 8.7 γ vitamin A per 100 ml. after this dietary change. This semi-synthetic milk contained ample choline.

These results showed that the increase occurred when fat, either butterfat or lard, was omitted from the diet and suggested that removal of the fat or some constituent associated with it (other than vitamin A) was responsible for the unexpected increase.

Four calves were changed from whole milk to skimmilk containing 1 to 2 g. of added choline chloride per day. No definite conclusion can be drawn from this

TABLE 2

Vitamin A values before and after various diet changes

	During vitamin A supplementation	1 wk. later on day of diet change	Days on ration indicated		
			1–2	3–4	5–8
When changed from whole milk to skimmilk + lard					
No. of calves	8	8	8	8	8
Plasma vitamin A (γ/*100 ml.*)	13.5	13.3	11.8	11.8	12.0
When changed from skimmilk + lard to skimmilk					
No. of determinations	.....	7	5	7	6
Plasma vitamin A (γ/*100 ml.*)	.....	12.1	12.2	16.6	15.9
Significance by *t*	.....	.....	n.s.[a]	*[b]	*
When changed from whole milk to skimmilk + choline					
No. of determinations	3	4	3	4	3
Plasma vitamin A (γ/*100 ml.*)	13.6	10.3	13.7	12.3	12.3

[a] n.s. = not significant, $P > 0.05$.
[b] * = significant, $P < 0.05$ but > 0.01.

small number of observations even though three out of four cases showed a definite increase. These average values also are shown in table 2 and suggest that the unexpected increase was not associated with the lowered intake of choline when skimmilk was fed.

Calf blood has been shown to contain factors that influence the Carr-Price reaction when the modified Kimble procedure is used to determine vitamin A in plasma (1, 12, 14). Since saponification removes or destroys these factors, vitamin A also was determined by a method used in this laboratory which employs total saponification and chromatographing (14). This method is a modification of the method used for milk and liver, and similar to the method used by Parrish *et al.* (12). Seven dietary changes from whole milk to skimmilk were made on four different calves and the vitamin A was determined by the two methods on a split sample of plasma. Both methods of determination showed that the plasma vitamin A level increased after the changes from whole milk to skimmilk and then decreased after the change back to whole milk. The changes in plasma

vitamin A level were of the same order of magnitude by either method of determination. The average values are shown in table 3.

Plasma carotenoid levels showed no unexpected increases or decreases during these dietary changes, as illustrated in figure 2. The values did indicate that the dietary intake of carotene was low. It was kept purposely low in these trials.

In these experiments the condition that brought about the increase in plasma vitamin A levels were: (a) the calves had a relatively large store of vitamin A; (b) the calves were changed from a diet containing carotene and/or vitamin A to one practically free of these nutrients; (c) the calves were changed from a diet containing butterfat or lard to a diet of a lower fat content and containing skimmilk; (d) one calf was changed from a synthetic diet to one free of fat and vitamin A.

An unexpected increase similar to that found in these experiments had been noted previously in young calves that were changed from colostrum to reconstituted skimmilk (4).

TABLE 3

Plasma vitamin A values before and after diet change when determined by modified Kimble and total saponification methods

	During supplementation	On day of ration change	Days on skimmilk				After 1 wk. changed back to whole milk
			1–2	3–4	5–8	9–14	
No. of determinations	1	7	2	6	5	4	3
Modified Kimble	21.8	10.7	9.1	13.3	15.5	12.3	8.5
Total saponification	23.5	14.3	13.6	17.9	19.6	13.6	14.3
$\frac{\text{Total sapon.}}{\text{mod. Kimble}} \times 100$ (ratio)	108	133	149	134	126	111	168

The substitution of lard for butterfat, which gave no increase in plasma values, indicates that factors other than the omission of carotene and/or vitamin A from the diet were responsible for the observed increase. Dietary fat or its related constituents was implicated in cases where: (a) skimmilk replaced whole milk; (b) skimmilk replaced the skimmilk-lard mixture; (c) fat and vitamin A were omitted from the semi-synthetic diet; and (d) a decrease was found when skimmilk was replaced by whole milk. The feeding of choline apparently did not prevent this unexpected increase in the absence of fat in the diet. The determination of plasma vitamin A by two methods indicates that the increase was not an artefact of the modified Kimble procedure and that it was a true increase in vitamin A level of the plasma.

It is possible that when the calf was receiving alfalfa or whole milk it used the dietary source to supply its daily needs for vitamin A. When the dietary source was omitted, then the body stores were called upon to supply the necessary vitamin A. The amount released and its concentration in the plasma may have been affected by any factor that might have altered the releasing mechanism. From the results obtained it might be suggested that skimmilk enhanced the release of vitamin A from storage, and that dietary fat or some factor associated

with it counteracted this mechanism. Blood fat levels and their relationships were not studied in these experiments.

The foregoing experiments show that under certain conditions the level of vitamin A in plasma was not a reliable indicator of the intake of the vitamin. These experiments were for only short periods of time, but on certain diets for longer periods of time a lack of correlation between intake and plasma levels also was noted (15).

SUMMARY

Experiments on young calves showed that dietary factors other than carotene or vitamin A intake affected the level of vitamin A in the blood plasma. When calves were placed on a diet deficient in carotene and vitamin A the level increased temporarily, for 4 to 24 days, and remained above levels that existed when on the adequate diet for 4 to 49 days.

In these cases the calves always had body stores of vitamin A and the deficient diet always contained skimmilk and a lower amount of fat than the adequate diet. Certain dietary factors apparently affect the release or utilization of vitamin A stored in the body. The results indicate that on certain dietary regimes plasma vitamin A levels were not a reliable indicator of intake.

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PLASMA AND STORAGE LEVELS OF VITAMIN A AND CAROTENE IN RELATION TO INTAKE BY CALVES ON DIFFERENT DIETS

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Literature on the relationships between plasma vitamin A levels and amount of vitamin A intake has been reviewed in a previous paper (3). Most of these reports indicated that there was a reasonable relationship between intake and plasma levels, but several inverse relationships were mentioned.

The results presented in the previous paper (3) showed that higher levels of plasma vitamin A sometimes occurred when calves were on a vitamin A-deficient diet than when they were on a diet adequate in vitamin A. Other factors in the diet were implicated for this unusual discrepancy. Since these trials were for short periods of time only, and liver storage of vitamin A was not investigated, it seemed desirable to perform experiments over a longer period of time and to obtain data on liver storage.

In this experiment the relationship of plasma and liver storage of vitamin A to the intake of vitamin A and carotene was determined for calves that were maintained on different types of rations for several months. Each calf was fed only one type of ration and level of intake. The experiments reported in this paper show that both the type of diet and the level of vitamin A intake are capable of affecting the relationships between the plasma and storage levels of carotene and vitamin A and the intake levels.

EXPERIMENTAL PROCEDURE

Different experimental and practical rations, with various levels of carotene or vitamin A, were fed to eight groups of young calves.

Group 1 consisted of six calves that had been depleted of carotene and vitamin A stores. They then were fed a grain mixture very low in carotene plus alfalfa meal to furnish a total intake of 26 γ of carotene per pound of body weight per day. Ten lb. of skimmilk also were fed to each calf daily. These calves averaged 346 days of age at slaughter and had been on this diet for 139 days when slaughtered.

Group 2 consisted of eight calves. They were fed a normal grain mixture, dried beet pulp, viosterol to furnish 3,000 I.U. of vitamin D per cwt. per day and carotene[1] to furnish 167 I.U. (100 γ) per lb. of body weight per day. They averaged 188 days of age at slaughter and had been on this diet since 10 days of age. Skimmilk was fed until the calves were 120 days of age.

Group 3 consisted of 11 calves. They were fed the same diet as group 2 except that they received no viosterol. Since both groups 2 and 3 were not allowed access to sunshine, this latter group became rachitic. They averaged 143 days of age when slaughtered.

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[1] A commercial concentrate containing 50,000 U.S.P. units per gram.

Group 4 consisted of four calves. They were fed a special dehydrated alfalfa hay and a normal grain mixture with skimmilk until 120 days of age. During this first 3 to 4 mo. when alfalfa consumption was low, they also received the same amount of the carotene concentrate as did the calves in groups 2 and 3. After this time their carotene source was the dehydrated alfalfa which occasionally was analyzed for carotene. They averaged 239 days of age when slaughtered.

One calf was treated and fed the same as the calves in group 1 except that, in place of the alfalfa meal, it received a daily capsule containing a vitamin A concentrate[2] to furnish a total intake of 25 I.U. of vitamin A per lb. of body weight per day. The calf had been on this intake for 176 days when it was slaughtered at 311 days of age. It will be referred to as group 5.

Groups 6, 7 and 8 were fed a semi-synthetic milk starting at 2 days of age and were held on this ration for a maximum of 90 days. A vitamin A concentrate[2] was fed by capsule to group 7 and was homogenized into the milk for group 8 to furnish 100 and 430 I.U., respectively, of vitamin A per pound of body weight per day. Group 6 received a special aqueous solution mixed with the milk to furnish 50 I.U. of vitamin A per pound body weight per day. Plasma vitamin A values on six, two and 16 calves and liver values on one, two and three calves in groups 6, 7 and 8, respectively, were obtained.

Plasma vitamin A determinations were made periodically; at slaughter, samples of plasma and the entire liver were obtained for carotene and vitamin A analyses. All analyses were done in duplicate. A modified Kimble procedure was employed for plasma and a total saponification and chromatographic procedure was used for liver (5). Carotene was determined in the rations by extracting according to the method of Moore and Ely (2) and chromatographing on $MgCO_3$ by a procedure adopted in this laboratory (5).

RESULTS AND DISCUSSION

A very good relationship between the carotene intake and the concentrations of carotene and vitamin A in the plasma and liver was found for the calves in groups 1, 2 and 4. These values are shown in table 1. Plasma carotenoid, liver carotene and liver vitamin A concentrations were linearly related to the carotene intake, while plasma vitamin A concentration showed a plateau as the intake increased. The results indicated that on a given constant intake of carotene the plasma carotenoid concentration may be a better indicator of carotene intake than plasma vitamin A concentration. Carotene and vitamin A concentrations in the liver also were a better indicator of intake than was plasma vitamin A. Liver vitamin A concentrations have previously been shown to be a better indicator of vitamin A intake than plasma vitamin A levels (1).

The relationship between carotene intake and plasma vitamin A levels was less evident for the calves in group 3. These calves became rachitic after their third month of life. Their carotene intake was the same as for group 2, yet their terminal plasma vitamin A concentrations were significantly ($P < 0.01$) lower

[2] Navitol with viosterol, containing 65,000 and 13,000 units of vitamins A and D, respectively, per gram.

TABLE 1

Vitamin A or carotene intakes and levels of vitamin A found in the plasma and liver of calves on various diets

Group no.	Diet	Daily intake/lb. of body weight		Average plasma vitamin A	Average liver vitamin A	Average plasma carotenoids	Average liver carotene
		Vitamin A	Carotene				
				(γ/*100 ml.*)	(γ/*g.*)	(γ/*100 ml.*)	(γ/*g.*)
1	Grain only	0	43 I.U. or 26 γ[a]	8.4 ± 3.6[b]	0.43 ± 0.3[b]	35	0.4
2	Grain and beet pulp and vit. D	0	167 I.U. or 100 γ	11.4 ± 5.8	2.1 ± 2.2	110	1.4
3	Grain and beet pulp no vit. D (rachitic)	0	167 I.U. or 100 γ	5.6 ± 2.2	2.0 ± 1.5	102	1.5
4	Grain and alfalfa	0	± 417 I.U. or 250 γ	12.6 ± 2.4	5.7 ± 4.4	245	4.1
5	Grain only	25 I.U	0	6.9	0.13	.....	.....
6	Semi-synthetic	50	0	4.1 ± 2.2	23.3	.....	.....
7	Semi-synthetic	100	0	4.8 ± 0.9	7.1 ± 2.0	.....	.....
8	Semi-synthetic	430	0	5.8 ± 2.0	40.0 ± 7.2	.....	.....

[a] = assuming 1 I.U. = 0.60α carotene.
[b] = standard deviation.

(5.6 compared to 11.4) than the values for animals on the same intake and ration but receiving vitamin D. However, the carotenoid concentrations in plasma and also vitamin A and carotene in the liver were the same for the two groups.

The plasma vitamin A values for these two groups were the same until the calves were over 60 days of age. After 90 days of age, the plasma vitamin A levels of the rachitic calves began to decrease, while the level in the calves of group 2 remained stationary or increased slightly. This divergence started at the time the calves began to show signs of rickets (4, 5) and progressed rapidly as the calves became older and more rachitic.

The above comparison is another instance in which it has been shown that dietary factors other than carotene intake can affect the level of vitamin A in the plasma. Similar observations were made previously (3). However, in the rachitic calves, liver storage of vitamin A and carotene was not affected. Further information concerning the effect of type of ration on plasma vitamin A concentration was obtained by comparing the values obtained for calves on semi-synthetic diets with those of calves on diets of natural feedstuffs, when both groups were fed vitamin A.

Plasma vitamin A concentrations of all calves fed a semi-synthetic milk diet were very low, being in the levels usually found in calves that are deficient in vitamin A. However, their liver stores were entirely adequate and very close to other published values for calves on comparable levels of intake (1). The plasma values of these calves showed very little relation to intake, as they showed only an insignificant increase of from 4.1 to 5.8 γ per 100 ml. when the intake was increased from 50 to 430 I.U. per pound daily. These values are shown in table 1 for groups 6, 7 and 8.

An entirely different relationship between intake of vitamin A and its concentration in the plasma and liver was obtained from calves in groups 6, 7 and 8 than was obtained from those in groups 1, 2 and 4. This is illustrated by comparing the two sections of figure 1. Possibly either the type of diet or the source of intake (carotene as opposed to vitamin A *per se*) may have been responsible for this difference. That type of diet is implicated as the major factor is indicated by comparing the values for the one calf listed as group 5 with those of calves on the semi-synthetic and near-normal diets (see table 1). This calf in group 5 was given vitamin A, *per se*, from the same source as groups 7 and 8, but at a low-intake level, and was maintained on a ration of natural feeds. Its daily intake was 25 I.U. of vitamin A per pound and its plasma and liver values are in line with those of the calves in group 1, which were on the same diet but were given carotene at 43 I.U. per pound. This calf given vitamin A at only 25 I.U. per pound per day consistently had higher plasma vitamin A levels than all but one of the calves that were on the semi-synthetic milk diet and receiving 430 I.U. per pound per day. However, its liver storage was negligible and significantly lower than the storage in calves receiving the semi-synthetic milk diet.

The plasma vitamin A values of calves on the semi-synthetic milk diet were much lower than values reported by others for calves on similar vitamin A intakes but on more normal rations, although liver stores were comparable (1). Since

the β-globulins in blood act as carriers for carotenoids, it is possible that the β-globulin level of these calves on the semi-synthetic milk diet was below normal. This might affect the plasma vitamin A levels.

These experiments also show some indication that the relation between intake and liver vitamin A concentrations was different when carotene was used as a source for vitamin A than when preformed vitamin A was fed. When approximately equal amounts, expressed as I.U. of carotene or vitamin A, were fed (groups 3 and 4 *vs.* 7 and 8) the liver store of vitamin A was much higher when vitamin A was fed (groups 7 and 8) than when carotene was fed (groups 3 and 4).

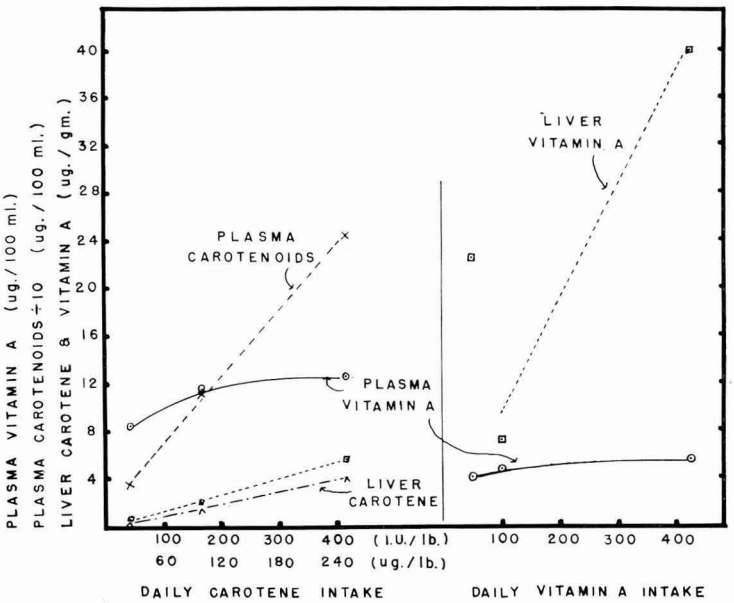

FIG. 1. The concentration of carotenoids and vitamin A in the blood plasma and livers of calves fed either carotene or vitamin A at comparable levels of intake. The figures in the left hand portion of the graph are from calves receiving natural feedstuffs and carotene (Groups 1, 2, and 4); figures in the right hand portion are from calves receiving a semi-synthetic milk and vitamin A (Groups 6, 7, and 8).

The above data are further proof that the relation between plasma vitamin A levels and intake levels can be altered by the type of diet. It was evident that plasma vitamin A concentration did not give a reasonable estimate of vitamin A intake of calves on the semi-synthetic milk diet nor of the carotene intake of rachitic calves. Neither did the plasma level give a reasonable measure of the liver storage on these diets.

The extent to which various dietary factors affect plasma vitamin A levels under usual dietary conditions where carotene is the main source of vitamin A activity has not been determined. In the one trial where carotene was fed at graded levels, the intake, plasma and storage values were directly related, but the

extent to which the plasma values had been modified by other dietary components was unknown. For practical purposes plasma values in this instance reflected intake and storage, but in establishing dietary requirements for vitamin A or in using plasma levels in a survey to estimate intake or storage the use of plasma levels may lead to erroneous conclusions if other dietary components exert a sizable influence on plasma vitamin A levels.

SUMMARY

On a ration of natural feedstuffs it was shown that the level of carotene intake of calves was linearly related to liver storage of both carotene and vitamin A and to plasma carotenoid concentrations. Plasma vitamin A concentrations reflected carotene intake until carotene intake reached a level of about four times the minimum requirement. Beyond this level no relationship was shown to exist.

In comparison with the above data, the measurement of plasma vitamin A concentrations did not give a reliable estimate of the carotene intake or of liver storage in rachitic calves. Also it did not give a reliable estimate of vitamin A intake or liver storage of calves on a semi-synthetic milk diet.

It is indicated that under certain conditions plasma vitamin A levels should be used with some degree of reservation as an indicator of vitamin A intake and storage until the effects of other dietary factors on plasma vitamin A levels are more thoroughly understood.

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CONCENTRATIONS OF CERTAIN MINERALS IN THE BLOOD AND LIVERS OF CATTLE AS RELATED TO TRACE MINERAL SUPPLEMENTATION AND BOVINE BRUCELLOSIS[1]

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In a study by Hart *et al.* (7) it was concluded that, "A high plane of nutrition, involving the feeding of alfalfa hay, minerals, cod liver oil and iodized salt, had no effect whatever in developing a resistance in cattle to *Brucella abortus*, as compared with a ration of lower protein content, supplemented with common salt, but no other minerals." More recently, however, some trace minerals which were not specifically considered by Hart *et al.* have been reported to have therapeutic value in both bovine brucellosis and undulant fever in humans (20).

The study reported herein was undertaken to determine whether cattle which were fed a practical dairy ration had lower concentrations of trace minerals in their tissues and organs than cattle which received a similar ration supplemented with trace minerals. A possible correlation between an active infection with *Br. abortus* and trace mineral concentrations in the livers of the cattle was also investigated.

EXPERIMENTAL

A large scale brucellosis study conducted at this station (3, 4) involved five experimental groups of approximately 20 Holstein cows per group. All groups were maintained throughout the experimental period on a practical dairy ration composed of either mixed legume hay or legume hay and corn silage, together with a grain mixture which was fed at the rate of 5 to 6 lb. daily, except in periods of lactation, during which time the cows received 10 to 12 lb. of grain per day. The percentage composition of the grain mixture was: whole oats, 19; corn or wheat, 48; bran, 17.5; soybean oil meal, 15; and iodized salt, 0.5. Only one of the groups of cows received a trace-mineral supplement which was mixed with 99 parts of the grain mixture to one part of a mineral mix composed of the following proportions of salts: Techmangam (product of Tennessee Eastman, containing 65 to 67 per cent $MnSO_4$, 100; $CuSo_4$, 1.0; $CoSO_4$, 1.5; $ZnSO_4$, 0.5; and $MgSO_4$, 3.0. Of the cattle which received no trace-mineral supplement, all except one group were vaccinated against brucella infection with strain 19 at various times. Cattle in the trace-mineral-supplemented group were not vaccinated. For details concerning the exposure of all animals to brucellosis by the instillation of 12×10^6 *Br. abortus* organisms in January, 1950, see Berman *et al.* (3).

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At the time of instillation all of the cattle had been receiving the experimental rations for 4.5 mo. or longer and most of them were in mid-pregnancy. Blood samples were obtained from the various groups prior to and following exposure. Liver samples were obtained from many of these animals at the time of slaughter, which was 12 to 18 mo. after exposure.

Blood analysis. A single aliquot of each blood sample, to which a small amount of solid sodium oxalate had been added as an anticoagulant, was ashed by heating with a mixture containing H_2SO_4, HNO_3 and $HClO_4$ acid for the colorimetric determinations of Fe, Cu and Zn with an Evelyn Colorimeter. The 2,2′-dipyridyl method of Jackson (8) was used for determining Fe. Cu was measured by the method of Clare *et al.* (5), with the slight modification that redistilled carbon tetrachloride was substituted for isoamyl alcohol to extract the Cu-diethyldithiocarbamate color. Zn was determined by the improved dithizone method of Vallee and Gibson (19). A. R. grade reagents were used throughout, and the water used in the determinations and for final rinsing of glassware was redistilled.

Liver analysis. Liver samples (about 3 lb. each) from the cattle were obtained, dried, powdered and stored in glass bottles. The wet ashing and general plan of analysis was based on the procedure of Parks *et al.* (14). A single 25-g. sample of each dry, powdered liver was digested using a total of 25 ml. redistilled water, 100 ml. redistilled HNO_3 and 25 ml. 60 per cent $HClO_4$ added in several portions as the digestion proceeded. Each ash was taken to dryness in a pyrex beaker on a hot plate and then dissolved in dilute, redistilled HCl. The volume was adjusted to 250 ml. with redistilled water or HCl in such a way that the final solution was approximately 0.6 *N*. These clear ash solutions were stored in glass-stoppered pyrex bottles. Aliquots were taken for duplicate analyses of Fe, Cu, Zn, Mn and Co by colorimetric methods. Some Mn analyses were made on 2.5-g. dry liver samples, which had been individually ashed in 100-ml. beakers, using H_2SO_4 in addition to HNO_3 and $HClO_4$.

Fe was determined by the ortho-phenanthroline method of Saywell and Cunningham (17), and Zn by the method of Vallee and Gibson (19). The method of Clare *et al.* (5) was used to measure Cu, except that isoamyl acetate was substituted for isoamyl alcohol, and water was removed from the acetate layer by centrifugation instead of filtration. Mn was determined by colorimetrically measuring permanganate according to Peech (15). Co was determined by the method of McNaught (12), after having first removed Cu and Fe as suggested by Kidson *et al.* (9). The final solutions of the Co-nitroso-R-salt were filtered through Whatman no. 40 filter paper just prior to being brought to volume for reading. Table 1 summarizes some pertinent data regarding the various methods used. All reagents used were A. R. grade, and the glassware was washed in soap solution, rinsed with distilled water, kept in 2 *N* HNO_3 6 hr. or longer and then rinsed with water redistilled from pyrex.

RESULTS

Analytical results are presented in summary form in tables 2 and 3.

Blood data. Just prior to inoculation with *Br. abortus* in January, 1950, the

average amounts of Fe and Cu in the blood of control (non-trace-mineral supplemented) animals and in the blood of trace-mineral supplemented animals were nearly the same. Likewise, the data on Zn in October and November, 1949, showed that the blood concentrations of Zn of the two groups differed very little.

The data on average Fe concentrations indicate that the blood of supplemented

TABLE 1

Methods used for liver analyses

Mineral	Vol. of ash soln.[a] used	Color-developing reagent	Vol. of soln. for colorimetry	Evelyn filter	Range[b]
	(*ml.*)		(*ml.*)		(γ)
Iron	0.5	*o*-phenanthroline	11	490	3–20
Copper	0.5	diethyldithiocarbamate	10	440[d]	3–25
Zinc	3.0	diphenylthiocarbazone	12[c]	520 and 620	4–60
Manganese	20.0	potassium periodate	11	520	15–100
Cobalt	90 to 100	Nitroso-R-salt	11	490	1–20

[a] Solution contained the ash from 25 g. of dry, powdered liver in 250 ml.
[b] Range of the amount of mineral for which the determination is suited.
[c] The 12 ml. is a 1: 6 dilution of CCl_4 of a 2-ml. aliquot taken from extractions brought to a volume of 50 ml.
[d] The 6-ml. aperture was used instead of the 10.

cattle contained slightly more Fe just prior to exposure to *Br. abortus* than 3 mo. later, in April.

Table 2 shows a range in concentration of 62 to 166 γ Cu per 100 ml. and 267 to 375 γ Zn per 100 ml. in the blood of the control cattle. Beck (1) reported 70 and 170 γ Cu per 100 ml. for normal cows, and Koga (10) reported approximately 440 γ Zn per 100 ml. beef blood.

In general, our data showed that there was as much variation within indi-

TABLE 2

Trace mineral concentrations in the blood of experimental and control cattle

Cattle group	Bleeding date	No. of animals	Fe (*mg./100 ml.*)		Cu (γ/*100 ml.*)		Zn (γ/*100 ml.*)	
			Mean	Range	Mean	Range	Mean	Range
Supplemented	Aug., '49[a]	18	38.0	32.5–42.5	107	69–160	318	237–470
"	Nov., '49	16[c]	41.1	38.2–45.5	123	80–171	311	264–362
"	Jan., '50[b]	19	46.9	42.0–54.5	114	87–282	.....	.....
"	Apr., '50	17	40.2	35.0–47.0	.....	.....	.....	.....
Controls	Oct., '49	11	37.3	31.0–42.5	93	65–110	319	267–375
"	Jan., '50[b]	14	45.1	40.5–50.0	115	62–166	.....	.....

[a] Samples were obtained prior to the trace-mineral supplementation period which was begun in Aug., 1949, and continued until the animals were slaughtered in 1951.
[b] All animals were exposed to infection with *Br. abortus* in Jan., 1950. January samples were taken prior to exposure.
[c] Zinc data are from only 7 animals.

viduals from time to time as there was between individual animals or between groups.

Liver data. Analyses showed that the concentrations of Fe, Cu, Zn and Mn in the liver were no greater in the trace-mineral-fed cattle than in control cattle. Some increase in the mean concentration of Co in the livers of trace-mineral-fed

TABLE 3

Trace mineral concentrations in the livers of experimental and control cattle

	No. of animals	No. vaccinated	All concentrations expressed as γ/g. dry liver powder									
			Fe		Cu		Zn		Mn		Co	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Supplemented	16	0	198	166–270	304	187–567	127	111–170	9.4	8.0–11.6	0.38	0.21–1.00
Infected	13	0	202	172–270	298	187–567	126	111–162	9.4	8.0–11.6	0.36	0.21–1.00
Uninfected	3	0	179	166–203	332	313–344	133	112–170	9.3	8.9– 9.7	0.46	0.37–0.59
Controls	14	13	208	138–332	299	165–578	124[a]	106–156	9.3	7.8–10.3	0.24	0.11–0.64
Infected	6	5	206	138–332	300	201–578	122[a]	114–131	8.8	7.8– 9.6	0.26	0.11–0.64
Uninfected	8	8	209	172–288	298	165–438	126	106–156	9.7	9.2–10.3	0.23	0.16–0.30
Infected (Sup. + Control)	19	5	203	138–332	298	187–578	125[a]	111–162	9.2	8.0–11.6	0.33	0.11–1.00
Uninfected (Sup. + Control)	11	8	201	166–288	308	165–438	128	106–170	9.6	8.9–10.3	0.29	0.16–0.59
Reported by others[b]	Ref.											
McNaught	(13)		302	137–644	49	6–191					0.24	0.12–0.40
Elvehjem *et al.*	(6)		294									
Kohler *et al.*	(11)			181 and 260								
Rost	(16)					To 393		To 277				
Koga	(10)						127					
Bentley *et al.*	(2)								9.5	6.4–23.0		

[a] These means are for 13 cows in the control group; 5 in infected, control; and 18 in infected (Sup. + Control).

[b] Concentrations were expressed in various ways by other authors and were converted to γ/g. dry beef liver for more direct comparisons.

cattle over the mean concentration in the livers of controls was indicated, but a wide variation existed within each group. By *t*-test statistical analysis (18), the difference between these means is not significant since there is a probability even greater than 10 per cent that the difference could be attributed to chance, *i.e.*, $P > 0.1$.

The data indicate clearly that there were no consequential differences in concentrations of Fe, Cu, Zn and Co in the livers of animals which had become infected with *Br. abortus* and those which had not. The difference between the mean Mn concentrations in the infected and uninfected control groups yields $P < 0.01$ in the *t*-test, but since duplicate Mn determinations in the quantitative range encountered are reproducible only within ± 5 per cent, the significance indicated by this test is doubtful. This view is further supported by the non-significant difference ($P > 0.1$) between the mean Mn concentrations in the livers of all the infected and uninfected animals.

Berman *et al.* (4) concluded that neither a measurable protection against a *Br. abortus* infection nor an acceleration in recovery from an active infection could be attributed to trace-mineral supplementation.

CONCLUSIONS

A practical ration which was used as a control ration apparently supplied abundant amounts of Cu, Zn, Co and Mn for Holstein cows, since dietary supplements of these trace minerals had little or no effect in changing the mean blood and liver concentrations in these animals.

Infection with *Br. abortus* in Holstein cows had no effect on the liver concentrations of Fe, Cu, Zn and Co and probably none on liver concentrations of Mn. The cattle which contracted induced Bang's disease had approximately the same concentrations of these five minerals in their livers as the cattle which were similarly exposed without becoming infected.

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THE ORIGIN OF SULFHYDRYL GROUPS IN MILK PROTEINS AND THEIR CONTRIBUTIONS TO "COOKED" FLAVOR[1]

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Many observations on the presence of sulfhydryl (-SH) groups have been made in conjunction with studies of so-called cooked flavor or the reducing system of milk (3, 4, 5, 6, 7, 9, 10, 11, 16, 18, 19). It has been demonstrated that cooked flavor first occurs when milk is heated momentarily to about 75° C. or at somewhat lower temperatures when holding periods are employed. Concomitant with the appearance of cooked flavor are a lowering of oxidation-reduction potential and the "liberation" of -SH groups and volatile sulfides in the milk (4, 7). Although all these phenomena appear to result either directly or indirectly from heat denaturation of certain milk proteins, a completely adequate explanation of their cause and relationship is yet forthcoming. The sources of -SH groups and volatile sulfides in milk have been shown to be the serum proteins, particularly the "albumin," and the proteinaceous material associated with the fat-globule membrane (4, 7). In connection with these latter studies it is important to note that β-lactoglobulin is the major component of the albumin fraction (14) and by reason of the quantity present in milk, fat globule membrane protein is a very minor source of -SH groups. This matter is further clarified by the work of Larson and Jenness (10, 11) which indicated β-lactoglobulin to be the principal reducing fraction of milk proteins and the primary source of -SH groups in milk.

Recent advances in protein chemistry have made possible a more detailed fractionation of milk proteins and the recovery of components having greater purity (13, 17). The main purpose of the present study was to determine the -SH content of a number of milk-protein fractions and to ascertain, in so far as possible, the contribution of these fractions to cooked flavor.

EXPERIMENTAL

Measurement of -SH groups. The method and apparatus employed for the titration of -SH groups were essentially those of Kolthoff and Harris (8), as adapted to the titration of -SH groups in blood serum proteins by Weissman *et al.* (20) and Benesch and Benesch (1). The principle of the method is as follows: When an -SH bearing protein is titrated in alcoholic, ammoniacal solution, with aqueous $AgNO_3$ at a rotating platinum electrode, against a suitable reference electrode, the insoluble silver mercaptide is precipitated and a negligible current flows until there is an excess of silver ions in solution. At this point the diffusion

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current of the silver ions at the rotating electrode rises sharply and in proportion to the concentration of these ions in solution. The end point is obtained graphically by plotting current readings against the volume of standard $AgNO_3$ solution added, and noting the point of intersection of the two straight lines. This method was chosen in preference to others because of its specificity for -SH groups. In addition, it was believed that the method could be employed satisfactorily in a quantitative determination of the -SH groups activated in milk by heat if alcohol were omitted from the titration mixture.

In preparing samples for titration the following procedure was adopted after careful study of the factors influencing the titration of skimmilk: Into a 100-ml. graduate were placed 1.25 g. of NH_4NO_3, 0.5 to 0.6 ml. of NH_4OH and sufficient distilled water to bring the volume to 21 ml. This mixture was placed in a 250-ml. beaker and nitrogen bubbled through the solution for 2 to 3 min. before 5.0 ml. of the sample to be titrated was added. The addition of 25 ml. of 95 per cent ethanol brought the final volume to approximately 50 ml. After the salt bridge and rotating platinum electrode were immersed in the solution, the titration was carried out by observing and plotting the deflection of the galvanometer after each addition of titrant. The electrode was rotated at approximately 150 rpm. The 0.001 *M* $AgNO_3$ solution employed in the titration was standardized by the method of Parks and Lykken (15). Performance of the platinum electrode was checked before each series of determinations by titration of cysteine-HCl. The apparatus was considered to be operating satisfactorily when titration of the -SH groups of this compound indicated quantitative measurement.

Preparation of protein fractions. Serum protein fractions were prepared from raw skimmilk by the methods of McMeekin (12) and Polis *et al.* (17). A brief account of the fractionation procedure employed follows: Acid whey, produced by precipitation and removal of casein from skimmilk at pH 4.6, was adjusted to pH 6.0 and made 2.3 *M* with $(NH_4)_2\ SO_4$. Euglobulin and pseudoglobulin fractions were obtained by dialysis of the 2.3 *M* insoluble fraction. The acid whey was increased in $(NH_4)_2\ SO_4$ content to yield a 3.3 *M* insoluble fraction. This material was dialyzed free of salt, adjusted to pH 5.2, seeded with β-lactoglobulin crystals and dialyzed further for approximately 30 hr. The β-lactoglobulin which precipitated was recrystallized by solution in 0.1 *N* NaCl, adjustment to pH 5.2 and dialysis. The supernatant liquid, obtained from the 3.3 *M* insoluble fraction after removal of β-lactoglobulin, was fractionated further. Adjustment to pH 9.1 with sodium borate and 2.4 *M* and subsequently 2.6 *M* with $(NH_4)_2\ SO_4$ precipitated two additional components which were recovered by filtration and designated fractions I and II, respectively. The filtrate, resulting from recovery of fraction II, was adjusted to pH 5.0 and made 3.4 *M* with $(NH_4)_2\ SO_4$. The material precipitating under these conditions was filtered and designated fraction III.

Several additional skimmilk fractions were prepared. These included casein and whey by supercentrifuging raw skimmilk at 34,000 rpm. for 30 min.; total milk protein and protein-free milk serum, the first by dialysis of 400 ml. of raw skimmilk against two 5-gal. portions of distilled water during 24 hr., the second

by dialysis of 400 ml. of distilled water against two 5-gal. portions of raw skimmilk during 24 hr. These dialyses were carried out in Visking membranes at 1 to 2° C.

Five experienced observers were employed to ascertain the contribution of the major serum-protein fractions to cooked flavor. The nitroprusside test was conducted and standards for the test prepared as previously described (16).

RESULTS AND DISCUSSION

General origin of -SH groups. In order to determine the extent to which -SH groups are distributed between the milk protein and its serum, the -SH content of raw skimmilk, dialyzed skimmilk and protein-free milk serum were measured by the argentometric-amperometric titration. Values of 0.127, 0.104, 0.103 and 0.000 m. eq./l. of -SH groups as cysteine were obtained for raw skimmilk, skimmilk aged 24 hr., dialyzed skimmilk and protein-free milk serum, respectively. These results indicated the -SH content of skimmilk to be associated entirely with the proteins. The reduction in titratable -SH groups on aging was found characteristic of all milks examined. Further information on the distribution of -SH groups was obtained by an examination of casein and whey prepared by supercentrifuging skimmilk. Results for raw skimmilk, casein and whey in this experiment were 0.117, 0.000 and 0.127 m. eq./l. of -SH groups as cysteine. These data indicate that whey is the source of -SH groups and that casein is completely devoid of them. The slight discrepancy in values for skimmilk and whey cannot be explained on the basis of volumetric differences, since both the whey and casein were corrected to the volume of the original quantity of skimmilk. The physical forces of centrifuging were not anticipated as a significant variable. However, they may have influenced slightly the number of reactive -SH groups of the whey in the titration.

The -SH content of serum protein fractions. The preceding experiments have shown that both casein and protein-free milk serum contain no -SH groups but that skimmilk, dialyzed skimmilk and whey do contain such groups. These findings indicate the serum proteins to be the sole source of -SH groups in skimmilk. Similar results, with methods other than the argentometric-amperometric titration have been reported (11).

Of the milk serum proteins, it has been suggested that β-lactoglobulin is primarily responsible for the -SH content of normal milk (11). Since information on this subject is limited and pertains mainly to β-lactoglobulin, a number of serum protein fractions were prepared and their -SH contents determined. The data in table 1 show that practically all of the -SH-bearing proteins were recovered in the 3.3 M $(NH_4)_2\ SO_4$ insoluble fraction which accounted for approximately 70 per cent of the total serum protein recovered. Further refinement of this fraction revealed that β-lactoglobulin was primarily responsible for its high -SH content. However, fraction III also contained a relatively high concentration of -SH groups. In order to ascertain the composition of this fraction, as well as to check the purity of the β-lactoglobulin, both materials were subjected to electrophoretic analysis. The β-lactoglobulin was found to be electrophoreti-

cally homogeneous at pH 8.4. Fraction III was composed of 40.4 per cent component C (13), 45.5 per cent β-lactoglobulin and 14.1 per cent true milk albumin (17). On the basis of these data, β-lactoglobulin is the primary source of -SH groups in normal skimmilk.

The effect of major whey protein fractions on cooked flavor. When it is considered that β-lactoglobulin represents 55 to 60 per cent of the milk serum proteins (13, 14), it alone would account for all of the -SH groups in skimmilk detectable by argentometric-amperometric titration. This might suggest that it is also the serum protein primarily responsible for the cooked flavor defect of heated milk. In order to test this contention, the following experiment was conducted. Lyophilized portions of each of the major protein fractions isolated from whey were added to skimmilk in an amount sufficient to double their normal concentrations. The -SH content of each sample was determined by ampero-

TABLE 1

The amounts and sulfhydryl contents of the serum protein fractions isolated from raw whey

Whey protein fraction	Amounts of protein fractions isolated[a]	Per cent of total protein recovered[b]	Sulfhydryl content of serum protein fraction expressed as cysteine
	(*g.*)	(%)	(%)
2.3 *M* $(NH_4)_2 SO_4$ (insoluble)	84.7	30.5	0.02
Pseudoglobulin	48.5	17.5	0.02
Euglobulin	31.8	11.4	0.02
3.3 *M* $(NH_4)_2 SO_4$ (insoluble)	193.2	69.6	0.32
β-lactoglobulin	98.1	35.4	0.53[c]
3.3 *M* $(NH_4)_2 SO_4$ (insoluble)			
Minus β-lactoglobulin	82.0	29.5	0.10
Fraction I	13.4	4.8	0.03
Fraction II	17.7	6.4	0.09
Fraction III	30.6	11.0	0.20

[a] From 50 l. of raw whey.
[b] Based on total proteins recovered in the 2.3 *M* (insoluble) and 3.3 *M* (insoluble) fractions.
[c] Recrystallized β-lactoglobulin.

metric titration prior to heat treatment. Following heat treatment at 82.2° C. (180° F.) for 20 min., the samples were subjected to organoleptic evaluation and the nitroprusside test. The results of this experiment are reported in table 2. The most outstanding feature of these results appears to be the pronounced effect of β-lactoglobulin on the development of cooked flavor. In addition it should be noted that cooked flavor and the nitroprusside reaction are closely correlated with the -SH content measured prior to heat treatment. This suggests that the argentometric-amperometric titration of skimmilk actually may determine the more readily accessible heat-labile -SH groups within the protein particle.

In amplification of this point, data are presented in figure 1 showing the differences in value obtained with and without alcohol in the amperometric titration of skimmilk samples given various heat treatments. These data show that titration in aqueous medium virtually coincides with that in alcoholic medium when the point of maximum -SH value in alcohol-free titration is reached. It is quite reasonable to expect that the holding times employed, despite flushing with

TABLE 2

Effect of doubling the normal concentration of certain major serum protein fractions in skimmilk on cooked flavor,[a] nitroprusside reaction and amperometric titration

Protein added	Per cent of total whey protein	Increase in whey protein fraction of skimmilk	Sulfhydryl content[e] of sample expressed as cysteine	Nitroprusside reaction	Degree of cooked flavor[b]
	(%)	(%)	(*m. eq./l.*)		
Skimmilk (control)	– – –	– – –	0.106	3	+
Pseudoglobulin	17.5[c]	0.12	0.111	3	+
Euglobulin	11.4[c]	0.08	0.108	3	+
β-lactoglobulin	55.0[d]	0.38	0.227	6	++++
Fraction III	11.0[c]	0.08	0.115	3	++

[a] Imparted by uniform heat treatment of all samples at 82.2° C. (180° F.) for 20 min.
[b] + = slight; ++ = definite; +++ = pronounced; ++++ = very pronounced.
[c] Approximations from whey protein fractionation data (table 1).
[d] Value obtained from literature and based on electrophoretic analyses (13, 14).
[e] Titrations conducted prior to heating.

nitrogen, would promote loss of some -SH content. This has been noted by Harland and Ashworth (5) and should be particularly true at elevated temperatures. Nitroprusside reactions of the heated samples also were observed. The color intensities of these correlated closely with the amperometric titrations in aqueous medium. Under these conditions, the two reagents, $AgNO_3$ and sodium nitro-

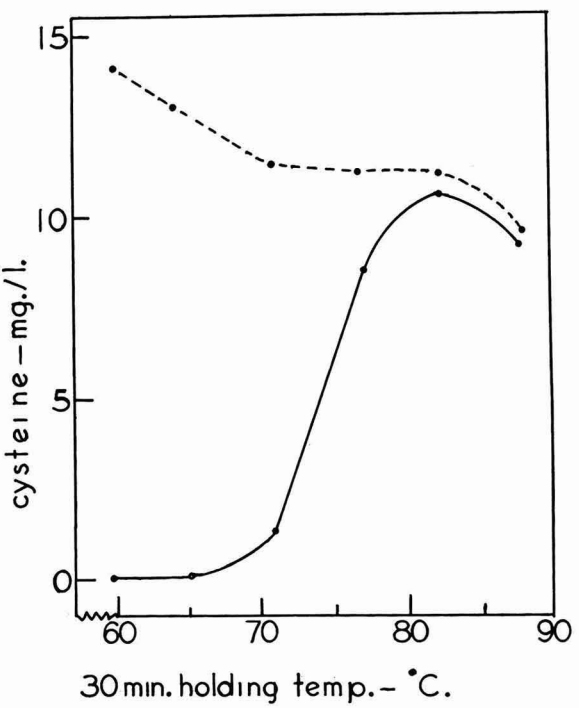

FIG. 1. Argentometric-Amperometric titration of heated skimmilk in alcoholic (– – –) and aqueous (————) media.

prusside, presumably measure the same -SH groups. Moreover, it seems probable that the argentometric-amperometric titration in alcoholic medium measures the total number of -SH groups in skimmilk which may be activated by heat. In this connection, it is interesting to note that the 0.127 m.eq./l. of cysteine (20.0 mg./l. of cysteine-HCl) obtained as a representative value of -SH content in raw skimmilk by the method reported, agrees closely with the 20.9 mg./l. of cysteine-HCl found by Harland and Ashworth (5) for heated (97° C. for 5 min.) skimmilk with the thiamin disulfide method. Slightly less than one-half of the -SH groups detectable in skimmilk by *o*-iodosobenzoate (11) are titrated in the argentometric procedure. The same situation is noted in the titration of crystalline β-lactoglobulin which has been reported to contain from 1.11 (2) to 1.30 (11) per cent cysteine. However, the 0.53 per cent cysteine obtained in this study agrees with 0.55 per cent found for β-lactoglobulin by others (12).

SUMMARY

Use of an argentometric-amperometric titration procedure has revealed that the only source of -SH groups in skimmilk is the serum proteins. Casein and protein-free milk serum were found devoid of such groups. Fractionation of the serum protein material into a number of components by $(NH_4)_2\ SO_4$ additions and pH adjustments revealed that β-lactoglobulin can account for practically all the -SH groups present. Study of the contributions of various major serum protein fractions to heat-induced cooked flavor in skimmilk demonstrated β-lactoglobulin to be responsible for the flavor. Conversion of -SH groups to H_2S as a result of heat treatment may explain, in a general way, the mechanism whereby β-lactoglobulin gives rise to cooked flavor.

The $AgNO_3$ titration, when conducted in aqueous medium, appears to measure the same quantity of -SH groups in heated milk as nitroprusside and thiamin disulfide. When conducted in alcoholic medium the total number of -SH groups capable of activation by heat treatment presumably can be determined in unheated skimmilk. The argentometric-amperometric method gives values for -SH content of slightly less than half of those obtained with *o*-iodosobenzoate for both skimmilk and β-lactoglobulin.

ACKNOWLEDGMENTS

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THE ANTIGENICITY OF BOVINE SPERMATOZOA

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That spermatozoa exhibit antigenic characteristics has been previously well described by Henle (5) and Henle *et al.* (6) in their work with the parenteral injections into rabbits of whole sperm and supersonically separated heads and tails of sperm. Landsteiner and Levine (7) also have demonstrated the antigenic activity of spermatozoa by showing that the sperm cells of humans of the appropriate blood type would adsorb specifically and almost completely the immune antibodies (from rabbits) to the A and B antigens of human erythrocytes. Cooper (2) reported from studies of the common leopard frog that antigens closely related to or identical with those of adult sperm were present in the eggs, embryos and larvae. The work of Burke *et al.* (1) established that demonstrable amounts of antigens exist in chick embryos which resemble the antigens of the adult sex organs.

The results reported in this paper deal with the development of techniques by which the antigens recognized in bovine blood cells by Ferguson (3), Ferguson *et al.* (4) and Stormont (8) can be recognized in the spermatozoa of bulls.

METHODS

Semen samples were obtained at intervals during this study from certain bulls through the cooperation of The Central Ohio Breeding Association. The semen was centrifuged and the fluid discarded, after which the spermatozoa were washed three times in 0.9 per cent saline solution. Following the last centrifugation, the supernatant was discarded and the packed spermatozoa were resuspended in saline solution to make a 5 per cent suspension.

Blood samples from the bulls, and from other animals used in certain of the tests to be described, were collected from the jugular vein in 3.5 per cent sodium citrate solution. The erythrocyte suspensions used in the tests were prepared by washing the cells three times in 0.9 per cent saline solution, after which the cells were resuspended in saline to make a 3 per cent suspension.

The lytic test, which was used to determine the antigens present in the blood, was that described by Ferguson (3) and used routinely in this laboratory in the blood typing of cattle. This technique consists of mixing 0.1 ml. of serum reagent for a particular antigen, 0.05 ml. of the erythrocyte suspension to be tested and 0.05 ml. of fresh rabbit serum as a source of complement. Thirty-two of the serum reagents, each prepared from isoimmune serum and each containing antibodies for a single antigenic component of the bovine cell, were used in this study.

Preliminary trials using the methods of Henle *et al.* (6) indicated that the

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spermatozoa were agglutinated specifically by the serum reagents but that the reactions were apparent only upon careful microscopic examination. In an attempt to obtain a more critical method of demonstrating the specific antigen-antibody reaction, an indirect method was developed using the lytic test as an indicator system.

The technique finally adopted was as follows: 0.05 ml. of the sperm suspension, diluted approximately 1:64 in 0.9 per cent saline, was added to 0.1 ml. of the serum reagent. This mixture was incubated 30 min. at room temperature (25 to 28° C). To this mixture was added 0.05 ml. of suspension of red blood cells, known from previous blood typing to be reactive with this particular serum reagent, and 0.05 ml. of fresh rabbit serum as complement. A parallel tube containing the serum reagent, the same red blood cell suspension and complement served as a control. The amount of lysis was recorded after 0.5, 1.5 and 3 hr. Controls on the complement and saline with each blood cell suspension were included in each test.

Repeatable results required a careful standardization of all materials used in the test. A dilution of each serum reagent was used which contained just enough antibody to produce complete or nearly complete lysis of the cells in a lytic test. Because the spermatozoa were anticomplementary in the more concentrated suspensions, it was necessary to determine by titration, prior to each test, the lowest dilution of spermatozoa which exerted no inhibitory effect on the complement.

Young, healthy sheep were used in a study to determine the antigenicity of bull spermatozoa. Ten ml. of a 5 per cent suspension of washed bull spermatozoa were injected intravenously in a ewe at weekly intervals for eight consecutive injections. The immune serum was collected from the ewe 3 days following the last injection and was stored at −20° C.

RESULTS AND DISCUSSION

Three bulls were chosen for these studies on the basis of the regular availability of semen for the detailed studies to be described. The antigens present in the blood of each of these bulls were as follows:

Bull 1—A, B, F, H, O, V, W, Z, E′, I′, M′, 11, and 12
Bull 2—A, B, F, G, H, P, W, Y, Z, C′, E′, M′, 2, 3, 6, 10, and 12
Bull 3—A, C, E, G, J, Y, Z, C′, E′, M′, and 6

The spermatozoa of each of these bulls were examined by means of the inhibition test and the following antigens were demonstrated:

Bull 1—A, B, F, H, O (Antigens beyond O were not determined since additional semen was not available.)
Bull 2—A, B, G, P, Y, Z, C′, E′, M′, 2, 3, 6, 10, and 12
Bull 3—A, C, E, G, J, Z, C′, E′, M′, and 6

A comparison of the antigens demonstrated in the blood and in the spermatozoa of each bull reveals a close correlation. Although the tests were not completed on bull 1, there was agreement for each antigen, except that variable results were

obtained with reagent for antigen H. These results can be explained by the fact that this reagent uniformly produces weak, incomplete serological reactions.

Although antigens F, H and W were demonstrated in the blood of bull 2, they were not found in the spermatozoa. The failure to demonstrate antigen H may have resulted from the weak nature of the serum reagent. An explanation of the absence of antigens F and W in the spermatozoa is not apparent.

Antigen Y was not found in the spermatozoa of bull 3, even though it was present in the blood cells. Except for this apparent discrepancy there was agreement for all the other antigens.

A comparison of the blood test results and the results of the spermatozoa inhibition tests shows that there were some antigens on the red blood cells which could not be demonstrated on the spermatozoa. However, no antigens could be demonstrated on the spermatozoa which were not present, also, on the red blood cells.

Several of the reactions were checked, particularly some of the systems which gave variable results, by means of antibody-adsorption tests. The serum reagent was mixed with washed spermatozoa and after 30 min. the spermatozoa were removed by centrifugation. The adsorbed serum reagent then was tested in a routine lytic test with a blood known to have the antigen in question. The results of these tests showed that bovine spermatozoa adsorbed only those antibodies which were inactivated in the inhibition test. In no instance did the spermatozoa adsorb a regent which it did not inhibit. These results add additional weight to the validity of the inhibition test.

The antigenicity of bull spermatozoa was demonstrated by immunizing sheep with the washed spematozoa of bull 2. There are certain antigenic components present in the erythrocytes of sheep which are similar to or identical with those in bovine blood. The blood cells of the sheep used in this immunization were typed and a comparison of the components of the sheep cells and those of bull 2 indicated that antibodies for antigens B, F, G, P, W, Z, C′, E′, M′, 3, 6, and 12 might be expected. Antibodies were demonstrated in the sheep serum by a microscopic agglutination test with bull spermatozoa. Also, antibodies were demonstrated in a hemolytic test using the sheep serum, bovine red blood cells and rabbit serum as complement. Further, the sheep serum, following adsorption with selected bovine erythrocytes, remained weakly reactive with certain bovine bloods. The results suggested that antibodies remained, in very low concentration, for antigens W and 3.

There have been reasons to suspect that the bovine cellular antigens might be present in other tissues of the body, just as antigens A and B of the human blood groups can be demonstrated in tissues other than the erythrocytes. This work shows that most of the bovine cellular antigens which were present on the red blood cells also were present on the spermatozoa.

SUMMARY

Iso-immune serum, containing antibodies for bovine erythrocytes, also reacted specifically with bovine spermatozoa.

Antibodies produced in sheep against bovine spermatozoa caused agglutination of bovine spermatozoa and also produced specific lysis of erythrocytes of certain cattle.

This evidence supports the theory that the antigens previously recognized in bovine erythrocytes have similar or identical counterparts in the spermatozoa.

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PROCEEDINGS OF THE FORTY-SEVENTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

P. R. ELLSWORTH, Secretary-Treasurer

The American Dairy Science Association assembled in the Victory Theatre, Davis, California, on June 24, 1952, at 10:00 a.m. Following the singing of the National Anthem, the invocation and an address of welcome by K. A. Ryerson, Dean, College of Agriculture, University of California, P. F. Sharp, presiding officer, introduced President H. A. Bendixen who gave the following address:

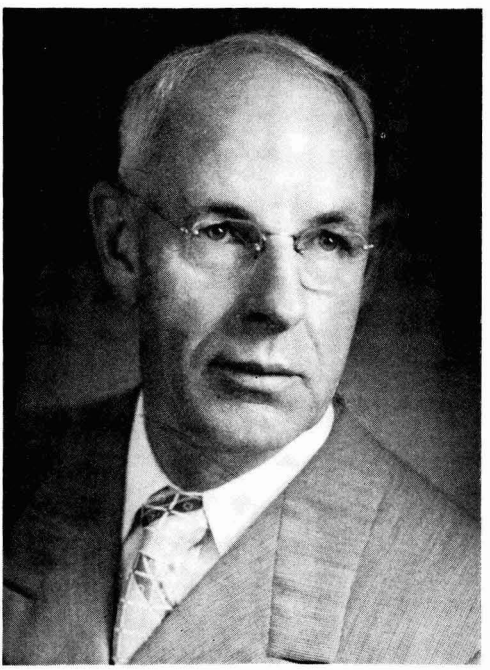
OUR ASSOCIATION, OUR INDUSTRY, OUR WORLD

It is indeed a pleasure to see such a fine representation of dairy scientists and friends of our Association assembled here at our opening session, in spite of the fact that we are meeting this year about as far away from the center of population of the continental United States as is possible. It appears that the magic word of California which electrified the world 100 yr. ago still retains its magnetic power, and all of us here today, I am sure, are thrilled to find ourselves under its spell. California called and, California, here we come. On behalf of every member present, let me thank you, Dean Ryerson, for your kind invitation and hearty welcome. We appreciate the tremendous effort put forth by the local committees on arrangements under the leadership of Dr. Jack and Professor Mead and to them and all others who have helped in planning the program and fine entertainment we express our sincere appreciation.

As dairymen we are indeed proud of the great progressive dairy industry of this state. California consistently leads the nation in production per cow. It ranks fourth among the states in total milk production, second in the production of evaporated milk, third in ice cream and first in sherbets and ice milk. It far outranks all other states in the production of cottage cheese, a product which was popularized and developed to its present high state of excellence in this area. California leads in innumerable ways. There is no denying California's just claim to fame for the magnificence of its mountains, its trees, its parks, its bridges, the variety of its flora, its climate and its people. Some have called it the land of the lemon, the prune and the nut and that perhaps is the reason why so many of us immediately feel at home and happy here.

Our Association. This is the 47th annual meeting of the American Dairy Science Association but only the fourth one to be held west of the continental divide and the third one to be held in California. It was 21 yr. ago when we last met here in Davis and the thought of it shocks some of us when we consider the changes that have occurred since 1931 in the industry and in ourselves. As in the old student refrain: "O jerum, jerum, jerum—o quae mutatio rerum" meaning in good American: "Oh, by jimminy, what a change!"

Now it is not my purpose today to detail to you the whole gamut of scientific and techno-



H. A. BENDIXEN

logical developments in dairying since 1931, no matter how proud we are of the record. If, however, a presidential address has any function at all, it seems to me, it is to critically reevaluate and redefine our Association's objectives and to ask the members to help rechart our Association's course as we round another corner on the road ahead. Where do we go from here? Are we properly and effectively discharging our responsibilities to our membership, our industry, our world?

The objects of our Association, as stated in our constitution are "to stimulate scientific research, to improve methods of dairy instruction and extension work, to cooperate in educational developments in advancing the general welfare of the dairy industry and to make available through the *Journal of Dairy Science* the results of the latest scientific research pertaining to these fields." Thus, our objects cover about every type of activity related to milk, man's noblest food, and its entire milieu from its mysterious origin to the equally obscure destiny of its products.

Our Association's responsibility to its members, as I see it, is to make it possible for them individually and collectively, to aid in the most effective manner in the activation of these aims. Thus, our annual conventions are designed to provide stimulation for the research man, the teacher, the extension specialist and the technologist. Here they may report on new findings, assess common problems, compare procedures, exert their leadership and initiate cooperative projects within Association committees and with industry groups, thus achieving a unity of purpose necessary for maximum success in the promotion of science and industry. Our plan of meeting in different parts of the country provides an invigorating experience for all of us. To maintain the purr in our human motors we need to occasionally take to the road. Just a change in atmosphere helps to recharge our spirit and to intensify our spark, and as we expose ourselves at our meetings to the infectious enthusiasm of others, as we applaud and are inspired by the recognition of outstanding accomplishments of fellow workers, as we reminisce with old friends and make new ones, and as we generally enjoy the geniality of kindred souls, we gain renewed faith in our calling and a kindlier feeling toward our fellow men.

An incidental service to our members that I would like to call to your attention, because it is being tried out for the first time this year, is an employment clearing service where prospective employers have an opportunity to list their openings, to interview applicants and to examine their credentials. I hope you will inquire about it in the Dairy Industry Department office and make use of it.

Research. The progress of mankind is accomplished largely through the two principal activities outlined in our Association's objects, namely, the constant search and research for new truths followed by the prompt and widespread dissemination of such truths. I cannot dwell here upon the proud record of scientific dairy research. Much, however, remains to be done.

In the scheme of God, the Almighty, man is so insignificant and his understanding of the world so limited that the discovery of one small fragment of knowledge here and there merely opens up another new awe-inspiring vista, and the solution of one small problem immediately poses another. No human being stands so humble in the sight of the handiwork of his Creator as the scientist. The astronomer reckoning the distance of the stars in terms of light years cannot possibly be a man superimbued with any thought of his own importance. The bacteriologist who for about a 100 yr. has been laboriously exploring the mysteries of a bewildering multiplicity of microscopic forms of life stands in overwhelming awe as suddenly with the development of the electron microscope an entirely new world of submicroscopic forms of life and matter opens up before his eyes. In this spirit of humility let us consider just a few of the problems challenging the dairy scientist today.

Production efficiency. Man constantly strives to raise his standard of living by increasing his production efficiency. The primary production machine of the dairy industry is the cow, and the efficiency of the cow may be increased by improving her breed, her feed and her management. That we are far from having reached maximum efficiency in the production of milk is clearly indicated by the tremendous difference still existing between the maximum and minimum amounts of milk produced per cow, per acre and per man and the maximum and minimum costs of producing a pound of milk solids in the United States. Just one illustration will suffice here. The average production per cow in 1951 was 7,700 lb. of milk and 300 lb. of fat in the State of California compared with 2,470 lb. of milk and 109 lb. of fat in another state. Then comparing these averages with nearly 42,000 lb. of milk and over 1,400 lb. of fat produced by the record-holding cows in the U. S. we realize how much may still be accomplished through increased production efficiency.

The most fruitful current development toward increased production is artificial insemination, which, while out of the experimental stage, still presents innumerable problems. We urgently need a dependable test for sperm fertility. We need to extend the *in vitro* life of the semen and learn more about the factors which influence embryonic death rates. What are the most effective systems of upbreeding our dairy animals for increased production? Too little is as yet known about inheritance factors by dairy cattle geneticists.

While the cow is already the most efficient converter of feeds unsuitable for human consumption, into milk, which is man's most nearly perfect food, dairy nutritionists today are still further

imposing upon the good nature of the cow by trying to induce her to utilize ever cheaper types of feeds such as straw and even wood products. I heard of a fellow putting green glasses on a mule and feeding him sawdust. The experiment was a success in that the mule ate the sawdust—but the mule died. The mule, however, is not blessed with a rumen as is the cow, and rumen bacteriologists are now hard at work trying to establish in the rumen of the patient cow types of bacteria which might assist her in breaking such cheap feeds down into useful food constituents. Even the calf, which should certainly be entitled to its mother's milk, is being made to partly give up milk and accept milk replacers or substitutes instead, so that human foster children may have more of the precious fluid.

Much can still be accomplished by the development of higher yielding varieties and mixtures of forages and by improving harvesting and storage techniques designed to retain maximum food values in these feeds. Pasture management still needs improving. Various diseases of dairy cattle, including brucellosis and ketosis are causing great losses to the industry. Building comfort as well as economy into shelters for cows and calves and their effect on the health and efficiency of the animals has only recently become the concern of our scientists.

Quality pays. Production line methods, which reduced the cost of automobiles, also are reducing the cost of milking and at the same time improving milk quality. Consumers are today buying more milk at higher prices, because they feel safer and happier drinking milk produced in a milking parlor instead of a barn.

On the other hand, grade A producers also enjoy the improved working conditions and are proud as peacocks about their facilities, although initially they cried to the high heavens because of the unreasonable demands of cursed milk sanitarians from the Public Health Service which, they felt, would most certainly bankrupt them. Yes, quality pays.

Unfortunately we also have the other side of the picture in the case of the cream producer for the butter industry. For 50 yr., ever since the introduction of the farm separator, we talked about the value of quality in cream for buttermaking and then accepted anything at the creamery that contained butterfat regardless of how it smelled. We talked about cream grades but did not ask for assistance from the Public Health Service to enforce them, and unfortunately for the butter industry the Public Health Service did not intervene. Our scientists developed effective salvaging techniques to convert a low grade raw material into a low grade or at best a mediocre spread. Today some sectors of the butter industry are neither proud nor prosperous and find themselves defenseless in competition with a product formerly considered of low birth, coming from across the tracks, and inflicted with a name which even those who buy it are ashamed to use at the dinner table. Mediocre quality and lack of uniformity of much of our American butter, however, made it easy for budget-conscious housewives to give up butter for the sake of guns for our soldiers. Let us help to return butter, with its naturally delicious flavor unimpaired, to America's dinner table because after all, bread and butter, and not bread and oleo, befit America's standard of living.

The two properties of milk which greatly complicate its transportation and merchandising are bulkiness and perishability. While aseptic canning is conquering the latter, research is attempting to correct both of these drawbacks of milk in one stroke by developing a powdered milk of high keeping quality. The stakes of developing such a product are high. Dried milk unimpaired in food value and palatability which might be easily reconstituted in the home would completely eliminate the expensive home delivery of fluid milk and would permit the cow to produce the largest amount and the highest quality of milk in the spring, which to her is doing what comes naturally. It would enable the farmer to practice summer dairying, usually more economical and certainly more pleasant than winter dairying. The housewife might manage with a smaller refrigerator, and milk would be available to loggers in the deep woods and to explorers or voyagers anywhere in the world. We would be able to ship a gallon of milk in a 1-lb. package from areas especially adapted to economical milk production to areas specializing in other types of agriculture or industry, to tropical lands where milk production is difficult and to arctic or other regions where man may go but cows refuse to work.

New products. Man always craves variety. New foods carrying new taste sensations are constantly coming on the market to compete with dairy products. While variety in ice cream is paying off, the possibilities of varieties of cheese which might be developed or are already known in other countries have not been exploited to the fullest by the American dairy industry. Some of our so-called by-products, such as buttermilk and whey, containing valuable food solids, still await the scientists' touch to give them glamor and taste appeal. They should find their way into a variety of food products, such as candies, soups, and bakery goods. Nonfat milk solids, just recently put on the retail market, are finding excellent acceptance and will be used in greatly in-

creased amounts as we teach housewives to use them.

Dairy manufacturing plants are streamlining their operations. Tank collection of milk from the farm represents a tremendous step forward in simplified procedure for both producer and plant operator. Ice cream plants are switching to the use of all-liquid raw materials and continuous processing. Continuous buttermaking is a reality the same as continuous operations in the evaporated and dried milk industries. Continuous cheesemaking is in the drawing board stage. All of these modern operations demand closer quality control and more technical supervision, making control laboratories, the eyes of management, more and more important. The shift of emphasis from fat to milk solids-not-fat makes imperative the emergence of another Dr. Babcock to develop a test for nonfat milk solids as simple as the Babcock test for fat.

Human problems. Our industry has its human problems too, and to render an all-inclusive service to our industry, it seems that our Association should include in its membership not only biological and physical scientists, but social scientists as well. We need the dairy economist to undertake time and motion studies, determine and analyze production costs, devise equitable pricing plans, study consumption trends, merchandising procedures, international trade possibilities and numerous other problems involving human action and reaction. We need a better understanding of the function of government in the production and sale of dairy products and should try to bring about happier relationships between producers and manufacturers, dealers and consumers, employers and employees and even between the many different organized groups within the dairy industry often pulling in different directions. All of us are illiterate in some fields. To serve our industry best we need to work together in teams, like specialists in a clinic. We need the social scientist and the social scientist in the dairy field needs us and all of us need to work closely with industry men to benefit from their advice.

Dairy instruction. Knowledge gained through research multiplies in value the more widely it is disseminated. An educated citizenry is fundamental to the success of every democratic organization and leadership toward progress in any important field is predicated on the acquisition of a great mass of accumulated basic information. A college education should supply this base. Poor teaching on the other hand is a betrayal of youth and an obstacle to human progress. What are we doing to improve dairy instruction?

This year our Association will receive the preliminary report of its curriculum committee effectively headed by past-president H. P. Davis of Nebraska. This report, the result of 2 yr. of diligent labor on the part of a large and representative group of educators in cooperation with industry leaders, will serve as a splendid guide for our college teaching staffs in outlining courses of study. Course outlines alone, however, do not insure effective teaching. Good teaching is an art as well as a science, a passion and a form of obsession. Consequently a good teacher is often more difficult to find than a good research man and rarely do we find combined in one individual the highest qualifications for both types of activities. Still, while teaching is the prime function of schools and colleges, it does not always receive the attention and recognition from adminstrators as does a sheaf of research publications.

How the world needs good teachers today, teachers able to train minds, to inspire, and to build vision, integrity, and moral fortitude into young personalities who are to be the leaders in research, in teaching, in extension, in industry, in public and community life! Just as the sire is half the herd so the teacher is half the school. We need proven teachers, but while we have developed procedures for proving sires, we have learned little about proving teachers. Many of our Association members have expressed the wish to have outstanding dairy teachers honored by awards similar to those bestowed upon our most successful research workers. Immediately, however, there arises the question of how to evaluate adequately the excellence of a teacher. A committee of this Association by action of your board yesterday will be set up to develop if at all possible a method for measuring the spark of those teachers able to set the minds and souls of their students afire with the fervent urge to serve not only themselves but all society. Yes, our Association's objective of improving dairy instruction is well taken and deserves increased activation.

Dairy extension. The members of our dairy extension section, who provide on-the-job dairy instruction in the field, develop each year at our convention a splendid program devoted to the improvement of extension teaching techniques. Could we not profitably devote some time at our meetings to the improvement of classroom teaching or have we long ago reached perfection in the instruction of our future leaders in dairy science and technology? Some of our classroom teachers might benefit more from sessions devoted to symposia on dairy instruction, teaching demonstrations and exhibits of modern teaching aids than they would from listening to certain research reports of a highly specialized nature and they might make valuable contributions of their own.

The American system of agricultural extension

education has been uniquely successful and many foreign countries today are eager to learn and to adopt similar procedures to help translate scientific discovery into practice. Most of our extension activities, however, have been in the production field. Is efficiency in plant operation less important than efficiency on the farm? Who suffers if the cheesemaker loses several vats of milk due to the presence of bacteriophage or antibiotics, if the ice cream plant is plagued with shrinkage problems, and the milk plant has flavor troubles? Dairy manufacturing specialists working for the improvement of quality and uniformity in all dairy products would benefit everyone from producer to consumer. They could be espcially helpful in improving public relations between producer and plant operators, between plant operators themselves, and between industry and consumers. They could also render most valuable service by coordinating the semi-educational work of milk sanitarians, dairy fieldmen, cow testers, 4-H workers, radio farm reporters, and even of sales representatives in the field. How much turmoil might be prevented if all could be made to speak a similar language? Let's have more dairy manufacturing extension workers.

The Journal of Dairy Science. Perhaps the greatest contribution of the American Dairy Science Association to the dessemination of scientific information in dairying is its *Journal of Dairy Science,* which today stands unexcelled in its field. Do not forget, however, that our ownership and publication of the *Journal* involves a great responsibility for our officers, the Executive Board, the Journal Management Committee, our Secretary, and especially our Editor. Due to sharply rising costs of publication we sustained unavoidable net losses in 1948, 1949 and 1950. A reduction in the net loss was achieved last year by an increase in dues and subscription rates. Now I am most happy to report that we were able to close the year 1951 with a profit balance of $1300. Credit for this fine showing is due to the able work of our Editor and Secretary. The latter increased our income from advertising alone by $2600 in 1951. The net worth of our association today is close to $30,000.

It behooves us, however, to remain alert to every possibility of improving the usefulness of our *Journal,* not only for our scientist members but also for our subscribers in the industry whose financial support of our association approximately equals that of our members. It is difficult for us to completely satisfy with one journal and a part-time editor the needs of our four types of clientele, namely the scientists in dairy production and in dairy manufacturing and the technological people in these two fields. People in industry, including members in our fine dairy technology societies and student affiliates, complain that half of the content of the *Journal* lies outside of their own specialty and many of the reports in their field of interest are written in too highly scienific language to be useful to them. Various requests have been received that our Association publish two publications, one scientific and one technological.

The Abstracts of Literature section of our *Journal* has been a problem child for the Association periodically, involving considerable time, worry and expense on the part of our Editor. Capable and prompt abstractors are hard to find and foreign literature and patent descriptions are rather inadequately covered at the present time. The abstracting service, however, was requested 16 yr. ago by several dairy industry associations, who formerly charged their members $5.00 per year for such abstracts, and many of our subscribers are especially interested in the abstracts.

What then should be our future course? Should we expand our publication to include a technological section containing information on new processes, new products, marketing trends, legislative matters, review articles and some news, together with enough advertising to help carry the load? Should we publish two journals, one scientific and one popular, or one dealing with dairy production and one with dairy manufacturing and then give each member his choice of one of the two publications? Should we expand our abstract section or drop it and ask a specialized abstracting journal to provide this service? In any event our Association has arrived at a point where perhaps two editors are needed to provide all the services demanded.

Furthermore, Dr. Nelson who for nearly 6 yr. now has carried a tremendous load as Editor of our *Journal* and has rendered us most outstanding service, wishes to retire at the end of this year and our Journal Management Committee has been searching diligently for someone to fill this important post.

These are all important problems which have been and still are engaging our attention. They are brought before you here because they should be the concern of every member in a democratic organization such as ours.

Our world. The publication of the *Journal of Dairy Science,* I am convinced, is the most important project of our association. Through it we serve not only our members and our industry, but the entire world, our world, which all mankind fervently hopes may someday exist as one world instead of two. The heartblood, which sustains the body of a free democratic society, is true information freely circulated to the ends of the world wherever free people are permitted to

seek the truths that might improve their own health and happiness. That we have iron curtains and tyrannical press, radio and thought controls in some parts of the world today should make us all the more determined to share our information with the free world to the fullest possible extent. This is not only our responsibility, but our sole hope of preserving our democratic way of life in a world, in which we now desperately need friends. Our nation in the past has been too preoccupied with its own economic progress to cultivate friends abroad. Today, however, the world has become so small in terms of distances that all people everywhere must definitely cooperate if we ever wish to enjoy world peace.

At the present time, for instance, our Board is considering an offer from the British Commonwealth Bureau of Dairy Science, which publishes Dairy Science Abstracts, to place their specialized abstracting service, subsidized by the British Commonwealth, at the disposal of our Association. Whether we can accept their offer will of course have to be weighed carefully in the light of our Association's finances and other considerations. I do feel, however, that we should fully explore every possibility of cooperating with our neighbors and avoid duplication of effort for the benefit of our dairy industry and theirs.

A similar situation exists in regard to our relationship with the International Dairy Federation, which sponsors the very valuable International Dairy Congresses. Some of the work of the I. D. F. may not be fully applicable to American conditions and the financing of delegates would be a definite problem for our Association. Still, maximum cooperation on our part with the scientists of the world in planning and staging the International Dairy Congress and attacking mutual problems would be to our definite advantage, not only from the standpoint of good public relations but for our protection as well.

For instance, Europe for years has been plagued with foot-and-mouth disease. Periodically we experience an invasion of this disease across our borders. Canada is under attack now, and we spent $100 million in Mexico alone to help control the disease there. What might have been accomplished with these funds applied to a united and coordinated world wide effort on a scientific front to eradicate this dreaded disease?

Science is not an American monopoly, nor a Russian one, though Soviet propaganda may try to convey that idea. Science is universal and we can profit greatly by a free interchange of ideas with dairy scientists across the seas. Let us not forget such famous names, familiar to all of us, as Van Leeuwenhoek of Holland, Lister of Great Britain, Pasteur of France, Koch and Weigmann of Germany, Segelcke, Stork and Orla-Jensen of Denmark, De Laval of Sweden, Virtanen of Finland, Gorini of Italy, Burri of Switzerland and many others. From Europe came the idea of cow testing, culture butter, the continuous separator, the pasteurizer, including the plate type machines, mechanical refrigeration, the homogenizer, continuous buttermaking, DDT and penicillin. Still to our colleagues abroad we have often given the appearance of standing aloof. We have made little effort to speak their language. We have restricted our trade with them when we should have lived up to the creed of the Yellow Dog "to gnaw in harmony that each may receive equal sustenance and pleasure." The isolation of Germany from world trade following World War I produced Hitler and World War II. Lack of cooperation with any part of the free world today may again invite dictatorships and precipitate World War III.

Recently I received an inquiry from the Allahabad Agricultural Institute of India asking about the possibility of establishing a student chapter of the American Dairy Science Association at that institution. What can we do for dairying in India and the millions of people in that country? In their new-found freedom they look toward America, the home of freedom, for guidance in their effort to banish the periodic specter of famine from their midst. Can we help them to increase the productivity of their land and their animals, including their sacred cows? They cannot buy our products; they do not want charity; they would welcome our helping them to help themselves. Certainly they will not readily give up the freedom for which they have struggled so long, but when hunger stalks the land, dictators readily gain control. The World Health Organization estimates that during the last 50 yr. the population of the world has increased by 826 million people and half of this increase was contributed by Asiatic people. Will these millions be for us or against us in our stand for freedom? Democracy can grow only among healthy and happy people. Just as dirty barns breed flies, so unhealthy economic conditions breed radicals and tyrants.

Let's guard too the health of our own economic conditions. Too many of us are still looking for a soft job with security or a racket in which mink coats and deep freezers just drop down our chimneys whether it is Christmas or not. We still want to believe in Uncle Sam as our Santa Claus to bring higher wages to all workers and support prices to farmers only to find that we must pay our Uncle back manyfold in taxes to support more bureaus, more inefficiencies, more scandals in public office, while strikes and turmoil continue on and on.

Many of you heard about MRA, Moral Re-Armament, at the Detroit Industries Convention. If not, read its inspiring story. It is one of the most hopeful signs of human progress in the social field. It has worked wonders in settling ruinous strikes here and abroad and has been an important factor in the reconciliation and up-building of Western Europe and the defeat of communism in that area. The crux of the MRA ideology is that all human disputes and problems may be dissolved if only we will agree to determine *what* is right instead of *who* is right. The fairness of such a creed is compelling. It will never again permit us to say: "America, right or wrong." It believes that modern science enables us to produce enough for everybody's need, but not enough for everybody's greed.

In a world in which over two-thirds of the population still goes continuously hungry we need a philosophy which calls upon all of us to care more and to share more. Let us do our part to increase human happiness wherever we can.

* * * *

Chairman Sharp then introduced Dr. Stafford L. Warren, Dean of the School of Medicine, University of California at Los Angeles who spoke on "The Cow in the Atomic Age."

The opening session adjourned at the conclusion of Dr. Warren's address.

BUSINESS MEETING
THE AMERICAN DAIRY SCIENCE ASSOCIATION
JUNE 26, 1952

President Bendixen called the Business Meeting to order at 3:00 p.m. in the Veterinary Science Auditorium. There were 120 members present.

REPORT OF THE EXTENSION SECTION

The Extension Section meeting was called to order at 1:45 p.m., Tuesday, June 24, in Room 100, Agricultural Engineering Building by Chairman Ramer Leighton. In the absence of Stanley Gaunt, Secretary, E. T. Itschner was elected secretary pro tem. On motion, reading of the minutes of the last meeting was omitted.

The following Nominating Committee was appointed: C. E. Gearhart, Pennsylvania; James G. Hayes, Michigan; Ivan H. Loughary, Washington.

Following brief general remarks by Chairman Leighton, the scheduled program on Teaching Methods and Exhibits was begun.

H. Thoele of Minnesota was introduced, and presented a paper entitled, "Identical twins in research," giving some of the methods and advantages of raising identical twins in research work. Additional comments were made by Lester Gilmore of Ohio.

A paper on "Visual aids in teaching through colored slides" was given by G. C. Anderson of Idaho. Illustrating his talk, Mr. Anderson used a series of colored slides developed over a period of years on the subject of "Irrigated pasture improvement."

Next a sound film in color was presented by Michigan State College on the subject "The right semen to produce the right calves," featuring the work of A. C. Baltzer and colleagues on artificial insemination.

Another film on "Weight reduction through diet," a production of the National Dairy Council, then was shown.

The group then adjourned to an adjoining room to review the state exhibits. Seventeen states had exhibits of teaching material and aids in conducting Dairy Extension projects. These included:

1. Use of I. B. Machines for handling D. H. I. A. records.
2. A manual for farm advisors on D. H. I. A. work.
3. Helps for D. H. I. A. supervisors.
4. Owner-sampler testing forms.
5. Breeding record cards.
6. Charts used in television shows.
7. Program planning manual for county agents.
8. Visual aids, and many others.

The meeting adjourned at 5:00 p.m. The attendance record showed 38 present, representing 22 states.

The Extension Section reconvened at 9:00 a.m., Wednesday, June 25th, with Ramer Leighton as chairman.

R. D. Stewart, of the American Guernsey Cattle Club reported for the P. D. C. A. Committee on a proposed "Uniform score card for 4-H judging, fitting and showmanship contests." The score card as presented was developed from score cards in use in various states and follows closely one used in New York State. Following discussion from the floor it was moved that the score card be referred to a committee to study, to present to extension workers in the states for their approval and suggestions and to make recommendations to the P. D. C. A. The motion was adopted. Later, Floyd Arnold, H. P. Ewalt and C. W. Nebbler were appointed on this committee.

G. E. Gordon of California gave a report on "The group system of judging and its merits." He outlined how group judging was used in California junior shows and pointed out its merits and limitations.

H. P. Ewalt of Oregon was introduced and in turn called on G. E. Gordon, of California, Ralph Erb of Washington, G. C. Anderson of Idaho and Lyman Rich of Utah, who outlined the status and development of artificial breeding work in their respective states. The reports show that in these states the following numbers of dairy cattle were being bred arificially: California, 81,000; Washington, 69,000; Idaho, 19,000; Utah, 28,000; Oregon, 39,000.

The group then again went into business session, and E. T. Itschner of Missouri was elected Secretary. Ivan E. Parkin of Pennsylvania, Vice Chairman, will move up as Chairman, and Stanley Gaunt of Massachusetts will become Vice Chairman. Committee reports then were presented and discussed. The session adjourned at noon.

The section reconvened in a joint session with the Production Section at 1:30 p.m., June 25. Chairman Leighton called for reports of the joint committees. These included: The Breeds Relation Committee—Hilton Boynton, New Hampshire; Dairy Cattle Health Committee—G. M. Werner, Wisconsin; Dairy Cattle Breeding Committee—C. D. McGrew, Ohio; Type Committee —I. W. Rupel, Texas; The Purebred Dairy Cattle Association—Floyd Johnston (for J. F. Cava-

All reports were adopted on motion.

The final phase of the joint session was the

Symposium on Bloat with N. N. Allen serving as chairman. This proved to be a most interesting naugh); and Antibiotics—read by N. N. Allen. and worthwhile session. Papers and discussions were given as follows: "The place of legumes in the pasture program"—R. E. Hodgson, B. D. I., Washington, D. C.; "The status of our fundamental information on bloat"—H. H. Cole, University of California; "Practical methods for prevention of bloat"—S. W. Mead, University of California; "Treatment of bloat"—G. H. Hart, University of California. Members of the panel responded to numerous questions during the discussion period that followed. The joint session adjourned at 4:00 p.m.

The extension Section met again at 9: 00 a.m., June 26th. Chairman Leighton opened the session and called on I. E. Parkin of Pennsylvania, the new Chairman, to preside.

L. H. Stinnett was introduced to preside during the period devoted to dairy records. The report of the Committee on Dairy Records was presented by members of the committee. It was in the nature of a general review of the D. H. I. A. system, its present status and recommendations for improving the service. This report was adopted. Chief O. E. Reed was present and was called upon for remarks. He reviewed the development of D. H. I. A. work and the increasing tie-up with Extension Service.

R. E. Hodgson discussed the program further in the light of limitations within the B. D. I. E. J. Perry then gave a paper on "D. H. I. A. regulations," reviewing some of the uniform rules and the need for close adherence to rules to maintain prestige of D. H. I. A. records. G. E. Gordon reported on D. H. I. A. work in California, followed by H. P. Ewalt for Oregon and L. H. Rich for Utah.

In the final afternoon session, Thursday, papers were presented by G. Heebink on "May the interpreters cooperate," dealing with the Extension Dairyman's responsibility in coordinating work of various agencies.

A paper was presented by G. E. Gordon on pipeline milkers, dealing with labor requirements of various types of milking systems.

The meeting adjourned at 3:00 p.m.

Respectfully submitted, Ramer Leighton, *Chairman;* Ivan Parkin, *Vice Chairman;* E. T. Itschner, *Secretary Pro tem.*

Upon motion duly seconded, the report was adopted.

REPORT OF THE MANUFACTURING SECTION

The Manufacturing Section held two symposia and four sessions, during which contributed papers were presented as listed in the official program of the Association. The symposia were presided over by E. L. Jack and other sessions by O. F. Garrett and A. J. Morris. Speakers in the symposia presented papers by invitation on the following subjects:

1. Some aspects of the effects of heat on milk.
2. The economic status of the dairy industry.

The business meetings were held on Wednesday, June 25, at 11:15 a.m. and 4:30 p.m. with Chairman E. L. Jack presiding.

J. H. Hetrick, Chairman of the Nominating Committee, presented a slate of candidates from which the following officers were elected for the ensuing year: G. H. Hartman, Secretary; A. J. Morris, Vice-Chairman; O. F. Garrett, Chairman.

Reports of the following committees were read and accepted as presented: 1. Uniform Procedures for Making Acidity Determinations of Fluid Dairy Products—J. G. Leeder, *Chairman;* 2. Judging of Dairy Products—G. M. Trout, *Chairman;* 3. Study of the Standard Plate Count with Particular Reference to Temperatures of Incubation—M. L. Speck, *Chairman* (Reported by E. B. Collins).

The above committees will be continued.

A report of the Association Standing Committee on Antibiotics in Milk was read by Chairman Jack.

The following committees did not report but will continue their activities for the coming year: 1. Standardization of the Babcock Test—E. O. Herreid, *Chairman;* 2. Standardization of Alkali Tests and Method of Reporting Results—D. H. Jacobsen, *Chairman;* 3. Evaluation of Methods for Determining the Activity of Cheese Cultures—H. C. Olson, *Chairman;* 4. Nomenclature and Methodology of Milk Proteins—A. M. Swanson, *Chairman.*

G. H. Wilster, Chairman of the committee on "The procedure and equipment for determining the fat in milk by the Babcock method," presented a written report which was accepted after revisions were made with reference to centrifuge speeds, directions on pipette specifications and use, and aliquot sampling. It also was voted to delete the last sentence of paragraph one which reads, "A mechanical stirring device shall be used with the weigh can, in order to satisfactorily mix the milk."

It is recommended by the section that Chairman Wilster submit the revised report to the Secretary of the section as a part of these minutes. The Committee will be continued to receive additional comments and suggestions concerning methods recommended in the report.

A written report of the Committee on Butter was made by G. H. Wilster, *Chairman.* C. A.

Iverson urged support of the 4-point program outlined in it. The section accepted the report with a recommendation to The American Dairy Science Association that the committee be authorized to activate and publicize its contents. This committee was voted continuation.

H. H. Sommer pointed out the importance of curd tension determinations, especially since the expanded use of reconstituted non-fat dry milk solids. A new committee was approved for the ensuing year to study the official methods for the determination of the curd tension of milk.

Respectfully submitted, E. L. Jack, *Chairman;* O. F. Garrett, *Vice Chairman;* A. J. Morris, *Secretary.*

On motion, duly seconded the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held four sessions for presentation of technical papers, at which 69 were presented. During each session two sections were run concurrently with chairman N. N. Allen and Secretary Philip L. Kelly, substituting for George Hyatt, Jr., Vice Chairman, presiding.

A business session was held at 11:30 a.m. on June 25. A report of the Pasture Investigation Committee and a letter from R. H. Lush, Chairman, was read. It was moved by I. W. Rupel and seconded that the report be approved and the committee having completed their work, be dismissed. The motion was passed.

A report of the Dairy Cattle Judging Committee, P. C. McGilliard, *Chairman,* was read. It was moved by Dwight Seath and seconded that the report be accepted. The motion was passed.

A report of the Resolutions Committee was presented by H. S. Willard, *Chairman.* After a motion for approval and an amendment by I. W. Rupel regarding the wording of the first resolution, the report of the Resolutions Committee as amended was passed.

A report of the Antibiotics Committee working in cooperation with the Manufacturing Section, W. A. Krienke, *Chairman,* was read. On motion by Rupel the report was approved.

It was moved by I. R. Jones, Chairman of the Nominating Committee, to elect George Hyatt, Jr., present Vice Chairman, to serve as Chairman and Philip L. Kelly, present Secretary, to serve as Vice Chairman the next year, as has been the previous custom. K. L. Turk moved that the nominations be closed and the vote be taken by a show of hands. The motion passed.

I. R. Jones then reported that the nominating committee recommended the names of R. E. Erb, R. E. Gardner and Eric Swanson for nomination as Secretary. Dwight Seath moved that the nominations be closed. This was seconded and R. E. Erb was elected Secretary.

Besides I. R. Jones, Ralph Hodgson and Glenn Salisbury were members of the Nominating Committee.

After remarks of appreciation by N. N. Allen for the fine cooperation he had received during his term in office the meeting was adjourned.

Respectfully submitted, N. N. Allen; George Hyatt, Jr.; Philip L. Kelly, *Secretary.*

Upon motion, duly seconded, the report was accepted.

REPORT OF THE REPRESENTATIVE OF THE AMERICAN DAIRY SCIENCE ASSOCIATION TO THE NATIONAL RESEARCH COUNCIL

It has been the experience of your representative that many members of scientific societies, including some actually engaged in research, do not have a clear understanding of the position and function of the National Research Council, nor of the relationship between their scientific societies and the Council. An introductory statement of clarification might, therefore, be appropriate.

The National Research Council is a cooperative organization of the scientific men of America. Its members include, however, not only scientific and technical men but also business men interested in engineering and industry. It was established in 1916 by the National Academy of Sciences and is supported by the cooperation of the major scientific and technical societies of the country. The membership of the Council is composed largely of appointed representatives of almost one hundred of these societies, and includes representatives also of certain other research organizations, representatives of Government scientific bureaus, and a limited number of members at large, numbering about 230 in all. These members receive their appointment from the President of the National Academy of Sciences.

Created in 1916 at the time of World War I at the request of the President, the Council was designated as the active agent of the Academy to assist the Government in organizing the scientific resources of the country. It soon became evident that the Council could perform useful peacetime functions as well, so at the request of the President of the Academy, President Wilson in 1918 issued an Executive Order which defined the duties of the National Research Council as follows:

1. In general, to stimulate research in the mathematical, physical and biological sciences, and in the application of these sciences to engi-

neering, agriculture, medicine and other useful arts, with the object of increasing knowledge, of strengthening the national defense, and of contributing in other ways to the public welfare.

2. To survey the larger possibilities of science, to formulate comprehensive projects of research, and to develop effective means of utilizing the scientific and technical resources of the country for dealing with these projects.

3. To promote cooperation in research, at home and abroad, in order to secure concentration of effort, minimize duplication, and stimulate progress; but in all cooperative undertakings to give encouragement to individual initiative, as fundamentally important to the advancement of science.

4. To serve as a means of bringing American and foreign investigators into active cooperation with the scientific and technical services of the War and Navy Departments and with those of the civil branches of the Government.

5. To direct the attention of scientific and technical investigators to the present importance of military and industrial problems in connection with the war, and to aid in the solution of these problems by organizing specific researches.

6. To gather and collate scientific and technical information, at home and abroad, in cooperation with governmental and other agencies, and to render such information available to duly accredited persons.

The administration of the Research Council is carried on by a small group of officers and an Executive Board. The Council itself is composed of eight major divisions, of which one concerns itself with international relations. The others are divisions of science and technology, representing respectively, physics, mathematics and astronomy; engineering and industrial research; chemistry and chemical technology; geology and geography; the medical sciences, biology and agriculture; and anthropology and psychology. With these divisions are associated various technical committees, appointed to take charge of projects undertaken by the Council. There are certain other committees, administrative and technical, which affiliate directly with the Executive Board of the Council. The Library of the Council, a limited collection of directories and source books in science, is available to the scientific public for reference services in so far as its facilities extend.

Financial support of the administrative work of the Council is derived from a gift of 5 million dollars to the National Academy of Sciences from the Carnegie Corporation of New York and from other special funds. A portion of this gift has been devoted to the erection of a building in Washington for the joint use of the Academy and the Council, but the greater part of the gift from the Corporation constitutes a permanent endowment in the hands of the Academy, the income from which is to be used for building maintenance and for the purposes of the National Research Council. The funds for the purchase of the land on which the headquarters building was erected were given to the Academy by about twenty friends of science. For the support of the scientific projects undertaken or sponsored by it the Council relies upon special gifts and appropriations obtained from time to time from various sources.

The Council is not an institution for the maintenance of scientific laboratories. It is rather an organization which, while clearly recognizing the indispensable value of individual investigation, hopes particularly to integrate the work of individual scientists and to assist in coordinating, in some measure, scientific attack in America upon large problems in the fields of scientific inquiry. The Council is perhaps implemented best for service in connection with problems which depend for successful solution on the cooperation of workers and laboratories within the realm of a single science or in the several realms in which various parts of a composite problem may lie. In most of these activities the function of the Council has been mainly to provide the auspices under which the scientific men of the country may join in the promotion of research. This is carried out through a wide variety of means, among which initiatory conferences have been found to be of especial value. Whatever has been accomplished by any of these means has been largely due to the personal contributions of American scientific men who, in association with the Council, have collaborated in these undertakings and have given generously of their time and effort for this purpose.

Within the structure of the National Research Council is the Division of Biology and Agriculture of which Dr. Paul A. Weiss, head of the Department of Zoology at the University of Chicago, is now Chairman. The activities of this Division of the Council concern most directly the membership of the American Dairy Science Association.

The Division of Biology and Agriculture has 30 affiliated scientific societies of which the American Dairy Science Association is one. There are, in addition, ten representatives of the Federal Government and five members at large. Three major bodies comprise the Division:

Agricultural Board—W. E. Krauss, *Chairman*
American Institute of Biological Sciences—T. C. Byerly, *Chairman*

Food and Nutrition Board—L. A. Maynard, *Chairman*

The following standing committees are also part of the Division:

American Type Culture Collection
Ecology of Animal Populations
Fellowships (N.R.C., N.S.R., and Fulbright)
Photobiology
Preservation of Indigenous Strains of Maize } To be affiliated with
Plant and Crop Ecology } Agr. Board
Use and Care of Natural Resources
Developmental Biology.

Activities of the Agricultural Board during the year most directly affect the interests of the American Dairy Science Association. The chief development affecting the future of this Board consisted of creation of an Agricultural Research Institute to be sponsored by the National Academy of Science. Following a meeting in Washington on December 10, 1951, at which approximately 100 representatives of agricultural industries, government agencies, experiment stations, research institutions, and farm organizations were present, an organization committee was appointed to work out the organization and fiscal procedures. This has been done and approved except for final editing. Under the proposed organization industry membership will be permitted at an annual fee of $300. Funds thus derived will be used to finance the activities of the Agricultural Board. Already numerous memberships have been obtained and the future activities of the Board seems to be assured through this needed financial support.

The following activities of the Agricultural Board are worthy of note:

Committee on Animal Health. Reports on losses of calves and young pigs are in process of preparation.

Committee on Animal Nutrition. The various subcommittees have been active in revising Recommended Nutrient Allowances for the various species of domestic animals and efforts have been directed through a specially appointed committee, to extend the circulation of these important texts for class room, nutrition conference, and general agricultural group use. Approximately 29,000 copies of these publications have been sold during the year. Four additional reports are nearing completion: Recommended Nutrient Allowances for Dogs, Fur Bearers, and Rabbits, and one on Use of Hormonal Materials in Animal Feeding. The American Veterinary Medical Association has requested a report on the use and dangers of antibiotics and growth-stimulating feed supplements in animal feeding. The problem of fluorine feeding will be reevaluated.

Committee on Feed Composition. Recent indications of financial support from the U. S. Department of Agriculture will stimulate this committee to complete its compilation of complete composition data on all feeds and to extend its study to include effects of soil, climate, drying temperature, etc., on the nutritive value or availability of feed nutrients.

Committee on Public Health Aspects of Brucellosis. A report on "Diagnostic criteria in human brucellosis" has been submitted for publication in the Journal of the American Veterinary Medical Association. This is the second in a series. The third will deal with the treatment of human brucellosis. The Proceedings of the Third Inter-American Congress on Brucellosis, held in November 1950 and organized by this Committee, are being distributed through the National Research Council.

The Committee on Laws, Rules and Regulations Governing Animal Health has submitted a 500-page typewritten report on state and federal legislation for the control of animal diseases and those transmissible to man. This is now being edited for publication as a National Research Council bulletin.

Committee on Milk Production, Distribution and Quality. Continuing its study under contract with the Dairy Branch of the Production and Marketing Administration under the Research and Marketing Act, this committee is now anticipating a final meeting to review the extensive manuscript and tabular material resulting from the second year's study of the relationships between sanitary laws and regulations and the quality of the market milk supply. Eight cities were studied: Rochester, New York; Boston, Massachusetts; Louisville, Kentucky; Houston, Texas; Birmingham, Alabama; Sacramento, California; Minneapolis, Minnesota; and Washington, D. C. The final report should have far-reaching significance.

While dealing with many problems of particular concern to humans, the *Food and Nutrition Board* has, through its Food Protection Committee, engaged in studies and the issuance of reports of general agricultural interest in that they deal with the important problem of residues resulting from the use of pesticides.

The American Institute of Biological Sciences is now well established but warrants the support of more affiliated societies. Preparation of the Handbook of Biological Data continues. The first volume dealing with blood has been completed and the volume dealing with nutrition is well under way. The Association and its membership should give careful consideration to lending active support to this worthy venture.

Applications for National Research Council,

National Science Foundation, and Fulbright Fellowships far exceed available funds. The most pertinent admonition that can be made for the benefit of those making application is to submit carefully documented credentials at least one year in advance of the time the candidate may be available for acceptance.

Your representative attended the annual meeting of the Division of Biology and Agriculture at the National Academy of Sciences in Washington on May 2, 1952. The above report is based largely on reports and discussion presented at that meeting, plus personal activities during the year as a member of the Executive Committee of the National Research Council, as Chairman of the Agricultural Board, as Chairman of the Committee on Milk Production, Distribution and Quality, and as a member of the Committee on Milk of the Food and Nutrition Board.

In the discussion at the annual meeting it was recommended that steps be taken to stimulate greater interest in the National Research Council among society memberships. Two ways that were considered helpful consisted of placing the report of the society representative to the Council on some place during the annual meeting of the society other than the business meeting, and to provide space in the society's journal for brief notes and reports pertaining to National Research Council activities.

Respectfully submitted, W. E. Krauss.

Upon motion duly seconded, the report was accepted.

EDITOR'S REPORT

Editorial material in the twelve issues of volume XXXIV which were printed during 1951 required 1,365 pages, even though the membership list was not included in this total because it was issued as a separate. Of the 159 manuscripts printed, 78 were on production subjects and 81 were on manufacturing subjects. Thirty-five manuscripts received during the year were rejected or withdrawn. Association announcements, lists of officers and committees, program of the annual meeting, abstracts of papers presented at the annual meeting and proceedings of the annual meeting required 92 pages. Indices and tables of contents required 47 pages, 20 of which were indices for the Abstracts of Literature section. The 706 abstracts printed in the Abstracts of Literature section required 109 pages. The use of less space between lines and smaller headings resulted in considerable increase in the amount of material which was printed on one page of this section.

Sixty-six manuscripts and 359 abstracts of the literature will appear in the first six issues of 1952; this compares with 77 manuscripts and 339 abstracts during the same period in 1951. The backlog of manuscripts for the last half of the current volume indicates that total publication probably will be almost the same as for last year. The Abstracts of Literature section is a little larger so far this year than last, but greater activity is desirable in that area. Several good reviews are in prospect for publication during the current year.

The editor wishes to thank all who have contributed of their time and energy for the maintenance of our publication. Those who serve as the anonymous reviewers deserve particular mention for the large part which they play in maintaining the standards of the *Journal*. A special word of appreciation is due to those who conscientiously prepare abstracts of the literature in order that the time spent by their fellow workers in following the literature may be kept at a considerably lower level than otherwise would be necessary.

G. H. Wise and P. R. Elliker have completed their terms as Associate Editors. Both of these men have made many valuable contributions to the success of the *Journal*; their counsel has been appreciated greatly. Both the Association and the Editor have been fortunate in having a Journal Management Committee the members of which have given much time and energy to the affairs of the *Journal*. J. K. Loosli retires this year from this Committee, to which he has contributed greatly. D. D. Deane prepared the index material for volume XXXIV and is continuing with the current volume. His assumption of this function has been most helpful. The fine assistance of Margaret Lange and Carolyn Nelson in handling many of the editorial duties, including proof reading and manuscript editing, is acknowledged.

At this time the Editor submits his resignation, effective December 31, 1952. The members of this Association have proven a fine group with which to work and the privilege of working with them has been appreciated. However, the pressure of other duties makes it necessary that the editorial duties be placed in other hands

Respectfully submitted, F. E. Nelson, Editor.

Upon motion, duly seconded, the report was accepted.

SECRETARY–TREASURER'S REPORT

Membership

The following is a summary of our gains and losses for 1951:

Membership, December 31, 1950	1703
Gains:	
New Members	97
Former Student Affiliates	27
Total Gain	124

Losses:		
Resigned	16	
Delinquent	208	
Deceased	5	
Total	229	
Net Membership loss		105
Membership, December 31, 1951		1598

Our membership loss, while high, is explained largely by the fact that dues were increased $2.00 per year in 1951. Such an increase will always result in a drop in membership and necessitates extra effort on the part of each member to bring in new members. In order to maintain any association a constant drive for members must be conducted. The normal forces of attrition alone will work to our definite disadvantage. Every member must assume the responsibility of urging others to join.

Our membership ranks were swelled by 27 former student affiliates who assumed full membership upon graduation from college. This number certainly justifies the plan of asking each Dairy Department to supply the Secretary with the names of graduating student affiliates. These graduates are then contacted by the Secretary and invited to assume full membership in our Association.

Every state in the Union is represented in our Association. The top four states in membership were New York with 156, Illinois with 148, Ohio with 115, and Wisconsin with 103.

Student Affiliates. A decrease of 410 student affiliate memberships occurred in the year just past. This decrease can be attributed to two factors: the student affiliate dues appear to be too high to attract undergraduates, and there is no national student affiliate program which could tie in with the parent organization and thus build interest and memberships.

At the present time there are 22 Student Branches of the American Dairy Science Association throughout the country engaged in varying degrees of A. D. S. A. activity. It does not seem unreasonable to set a goal of a Student Branch at each Dairy Department in the country. A membership of at least ten 4-yr. dairy majors meets the minimum requirements for a Student Branch. Certificates suitable for such branches can be obtained by contacting the Secretary.

Circulation. The circulation of the *Journal* reached 3,265. While the membership dropped, the number of subscribers increased. Approximately one-half of the circulation of the *Journal* is accounted for by subscriptions to institutions, companies, and individuals both in the United States and in 45 foreign countries.

Finances. During 1951 the *Journal* carried 110 pages of paid advertising which brought in a revenue of $7,204.05. This represents an increase of $2,597.10 over the previous year. Although the total number of pages of paid advertising remained practically the same as in 1950, the rate charged per page was increased effective January 1, 1951, and the increased revenue resulted therefrom. Those companies advertising in the *Journal* contribute much to the success of your Association and deserve your consideration whenever possible.

The following table shows the sources of revenue and items of major expense of your association during 1951.

Revenue	
Membership Dues	37.11%
Student Affiliate Dues	4.66%
Subscriptions	35.00%
Advertising	20.40%
Back Copy Sales	2.83%
Expenses	
Journal	74.80%
Editorial Office	10.57%
Secretarial Office	12.10%
Other	2.53%

There are indications that the cost of operating your Association will be higher in 1952 and 1953. In order to meet these increased costs, an active effort will be made to obtain more advertising, additional members anud student affiliates and a larger number of subscribers. The sincere cooperation of all will be needed if these goals are to be obtained.

Decennial index. Thanks to the untiring efforts of Dr. H. Macy, the Decennial Index covering volumes 21 through 30 is now ready for printing. Shortly after the annual meeting each member and subscriber will be sent an order form by means of which a copy of the index may be obtained. The demand for this index will determine the course of action with regard to future indices.

The Secretary wishes to take this opportunity to express his sincere appreciation to all officers, Board Members and members who have given so generously of their time and effort to see this Association through another year.

Respectfully submitted, P. R. Ellsworth, *Secretary-Treasurer.*

Upon motion, duly seconded, the report was accepted.

AUDITING COMMITTEE REPORT

To the Executive Board and Members of
The American Dairy Science Association
Gentlemen:

On June 6, 1952, Mr. Walter C. Burnham, a Certified Public Accountant met with the Auditing Committee of The American Dairy Science Association. At that time Mr. Burnham's report

of his audit of the Association's business for 1951 was considered.

Mr. Burnham has made a thorough examination of the records. He has checked the bank statements and examined all the U. S. Government Bonds. Mr. Burnham has check-tested the inventory of Journals and Twenty-Year Indices to assure accuracy of the physical inventory.

The Auditing Committee is satisfied that the financial statement for the year 1951 is correct. The committee wishes to commend Mr. Burnham, the auditor, for his fine work and excellent report. We recommend that the financial statement be accepted by the Executive Board and the members of The American Dairy Science Association.

Respectfully submitted, L. O. Gilmore; W. L. Slatter; T. S. Sutton, *Chairman.*

Upon motion, duly seconded, the report was accepted.

REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

During the past year the committee has acted on questions submitted by the Editor concerning routine matters of permission for authors to use material from the *Journal,* and on publication of certain papers of unusual nature or origin.

The committee has elected E. M. Foster and E. W. Swanson as Associate Editors to replace P. R. Elliker and G. H. Wise. The committee is grateful to the retiring Associate Editors for the years of faithful service they have donated to the Association.

In specific actions the committee has recommended:

1. That the Association membership list be published in booklet form and mailed only to members.

2. That the 10-yr. index for Volumes 21–30 be published by the Association and sold at a price to cover fully the publication costs.

3. That the idea of using British Dairy Science Abstracts instead of the Abstract Section of the *Journal of Dairy Science* be rejected.

4. That the abstract section of the *Journal* be expanded.

5. That consideration be given to publishing more material in the *Journal* to improve its service to men in industry; that such material might include reviews and discussions of current problems, and descriptions of processes, machines and significant inventions; and that the popular, non-technical style of writing be used in the preparation of such material.

6. That two publications, one of original papers, the other of abstracts, reviews and the like, might serve our membership eventually, but such expansion should not be attempted until the reactions of members and subscribers can be observed or tested.

7. That an editor and an assistant editor are needed to maintain the present standards of the *Journal,* to expand the scope of the abstract section and to develop the service of non-technical discussions and reviews.

These recommendations the committee respectfully submits to the Executive Board and asks for further instructions to proceed according to these recommendations or to abandon them and to maintain the *status quo.*

The Journal Management Committee wishes to express the commendation of the Association Membership to the Editor and the Editorial Staff for their continued excellent and untiring efforts.

The members of this committee extend their grateful thanks for the personal, friendly guidance of F. E. Nelson. His skillful, patient, and tactful handling of his editorial responsibilities has been a major factor in maintaining the excellence of the *Journal.* The members of this committee appreciate the necessity which compels him to resign from this position. They regret exceedingly the loss which the Association must sustain. This loss to the Association will be compensated by his direct contributions to the science of dairy bacteriology to which he can now devote his enthusiastic study and entire energy. Our best wishes go with him.

Respectfully submitted, R. E. Hodgson; W. V. Price; J. K. Loosli, *Chairman.*

Upon motion, duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE REPORT

WHEREAS: The 47th Annual Meeting of the American Dairy Science Association has been held on the campus of the University of California at Davis, and

WHEREAS: The host institution undertook the task and responsibility of arranging the details necessary for the successful conduct of the annual meeting, and

WHEREAS: The arrangements that have been made by the local institution have been more than adequate in every detail, thus permitting this Association to hold one of its most pleasant and successful meetings enjoyed by men, women and children alike, now

Therefore be it RESOLVED: That the American Dairy Science Association extend its sincere appreciation for the courtesies extended and the services provided for by the University of California, to Dr. Robert Gordon Sproul, President, University of California, to Dean K. A. Ryerson, Dean of the College of Agriculture, and especially

to Dr. E. L. Jack and Professor S. W. Mead, Co-Chairmen of the local committee and their associates who contributed so much toward making possible this successful meeting.

WHEREAS: Many commercial organizations have contributed greatly to the success and enjoyment of the 47th Annual Meeting,

Therefore, be it RESOLVED: That the American Dairy Science Association express to those organizations its sincere appreciation.

WHEREAS: The Borden Company Foundation has for the sixteenth year made available for presentation by the American Dairy Science Association the Borden Awards for outstanding research in dairy production and dairy manufacturing,

Therefore, be it RESOLVED: That the American Dairy Science Association express its appreciation to the Borden Company Foundation for these Borden Awards by which the American Dairy Science Association may recognize achievement in the fields of dairy production and dairy manufacturing research.

WHEREAS: The American Feed Manufacturers Association has again made available to the Association the American Feed Manufacturers Award for outstanding research in dairy cattle nutrition,

Therefore, be it RESOLVED: That the American Dairy Science Association express its appreciation to the American Feed Manufacturers Association for this award by which recognition is given to outstanding research in dairy cattle nutrition.

WHEREAS: The De Laval Separator Company has again made available an award for outstanding achievement in the field of dairy extension,

Therefore, be it RESOLVED: That the American Dairy Science Association express its sincere appreciation to the De Laval Separator Company for the award by which outstanding work in the field of dairy extension may be recognized.

WHEREAS: Proved sires are such an important factor in building high producing dairy herds and,

WHEREAS: Such sires are still not available in sufficient numbers for the best interests of the dairy industry,

Therefore, be it RESOLVED: That the American Dairy Science Association cooperate with the Bureau of Dairy Industry of the U. S. Department of Agriculture and encourage national support of the D.H.I.A. program, and

Be it further RESOLVED: That the breed associations be encouraged to send A. R. and H. I. R. records to the Bureau of Dairy Industry for use in proving sires.

WHEREAS: Dairy cattle disease, parasites, and injuries have been a source of great loss to dairy farming, and,

WHEREAS: It is recognized by the American Dairy Science Association that the economic advancement of the dairy industry is primarily dependent upon the solution of these problems,

Therefore, be it RESOLVED: That all agencies working in the field of dairy cattle health be commended for their activity in research and in the encouragement of education and research in preventing losses in livestock and the spread of disease.

WHEREAS: The National Research Council acts as an organization which assists in the solution of problems of the dairy industry, and,

WHEREAS: The National Research Council has several committees in Animal Nutrition and in other fields of agriculture, but no committee on animal breeding and genetics, and,

WHEREAS: There are many problems involved in dairy cattle breeding and genetics,

Therefore, be it RESOLVED: That the American Dairy Science Association recommend to the Agricultural Board of the Division of Biology and Agriculture of the National Research Council the establishment of a committee to consider problems of dairy cattle breeding and genetics.

WHEREAS: Much of the success and the influence of the American Dairy Science Association finds expression through the publication of the *Journal of Dairy Science,* and,

WHEREAS: During the past 6 yr. under the capable editorship of Dr. F. E. Nelson our journal has advanced its prestige, nationally and internationally,

Therefore, be it RESOLVED: That The American Dairy Science Association express its sincere thanks and appreciation to Dr. F. E. Nelson for his constant and successful efforts as Editor of the *Journal of Dairy Science.*

Respectfully submitted, A. O. Shaw, *Chairman;* G. E. Holm; A. H. Rishoi; H. S. Willard; C. N. Hall.

Upon motion, duly seconded, the report was accepted.

REGISTRATION COMMITTEE REPORT

Registration for the 47th Annual Meeting totaled 640, including 167 women and 102 children representing 42 states, the District of Columbia, Alaska, Hawaii and four foreign countries.

The top four states in attendance, excluding California, were Illinois, 73; Minnesota, 48; Wisconsin, 44; and Ohio, 40.

Respectfully submitted, C. A. Phillips, *Chairman.*

Upon motion duly seconded, the report was accepted.

EXECUTIVE BOARD MEETING

The Executive Board transacted the following business:

Approved the minutes of the 1951 Annual Meeting.

Approved the Editor's Report.

Accepted with regret the resignation of Editor F. E. Nelson effective December 31, 1952.

Approved the Secretary-Treasurer's Report.

Accepted the Journal Management Committee Report and approved the Journal Management Committee recommendations that the Association not accept the proposal of purchasing British Dairy Abstracts to replace the *Journal* abstracts. The Board approved the committee's recommendation that the abstract section of the *Journal* be expanded and that it include technological articles, reviews and other such material.

Approved the Auditing Committee Report.

Approved a Budget of $41,600 for 1953.

Approved the Resolutions Committee Report.

Elected J. H. Erb to the Journal Management Committee to serve for 3 yr. (1953–54–55).

Re-elected P. R. Ellsworth as Secretary-Treasurer for 1953.

Approved the selection of C. L. Roadhouse as Honorary Member.

Approved C. Albert Altwegg as Life Member.

Renewed Student Affiliate Branch Certificates for the University of Florida, University of Georgia, University of Massachusetts, Rutgers University, Oklahoma A & M College, University of Tennessee, Virginia Polytechnic Institute, and Ohio State University.

Approved the establishment of a Student Branch at the Allahabad Agricultural Institute, India.

Accepted, with thanks, the offer of the Borden Company Foundation to make the Borden Awards in Dairy Production and Dairy Manufacturing Research available in 1953.

Voted to recommend to the Association that the General Business Meeting be held from 10: 30 to 12: 00 on the morning of the third day of the Annual Meeting.

Appointed G. M. Trout the Association Historian with indefinite term of office.

Voted to recommend that the Association not join the International Dairy Federation, but rather that it support the World's Dairy Congress as now constituted or through a world dairy science association. The Board felt that the work conducted by the commissions of the International Dairy Federation could be better handled insofar as this country is concerned, on a national basis.

Accepted the report of W. E. Krauss, representative of the Association on the National Research Council, Division of Biology and Agriculture.

Accepted the progress report of the Curriculum Committee indicating much progress but no definite conclusions at this time.

Recommended the appointment of a committee to study the feasibility and mechanics of an Outstanding Teacher Award.

Accepted the invitations of Pennsylvania State College and the University of Connecticut to hold the annual meetings there in 1954 and 1956, respectively.

Accepted the report of the Pasture Improvement Committee and voted to terminate the committee.

Received the report of the Sustaining Membership Committee and voted to take no action at this time on the recommendations contained therein.

Voted to recommend to the Association that all *new* Student Affiliates paying their dues to the Secretary-Treasurer prior to November 1 of any year receive the next year's *Journal* plus the October, November, and December issues of the *Journal* of the year in which the dues were paid.

Voted to recommend that a permanent five man Committee on Student Affiliate Affairs be appointed. This committee to study the entire student affiliate program and draw up plans for a national student affiliate organization.

Respectfully submitted, P. R. Ellsworth, *Secretary.*

Upon motion, duly seconded, the report was accepted.

NECROLOGY COMMITTEE REPORT

During the calendar year 1951 the following members of the American Dairy Science Association passed on to their final reward.

Winfred Enos Ayres, Associate Professor Emeritus, Department of Dairy Industry, Cornell University, died on September 5, 1951, in Albany City Hospital following an operation. He was 68.

At his retirement in June, 1949, Professor Ayres had been at Cornell 40 yr. as resident teacher and extension worker. He possessed that exceptional ability as a teacher to develop the individual student. His patient and kindly attitude encouraged students to ask questions. His knowledge of dairy products enabled students to obtain accurate information, and his cheerful philosophy

gave many encouragement for their post-college days.

For several years before joining the Cornell staff, he was a creamery operator. From 1906 to 1910, he taught in the Winter Dairy Course and did extension work among dairy plants. Following this, he became a state butter inspector in the New York State Department of Agriculture and Markets. In 1913 he entered the service of the Vermont State Department of Agriculture as a dairy plant inspector. In 1914 Cornell asked him to take charge of the Winter Dairy Course and later to teach the manufacturing of milk products to 4-yr. course students. Through his extensive knowledge of the quality of dairy products, he helped train many good teams in the judging of dairy products.

He was a member of the American Dairy Science Association and Epsilon Sigma Phi, honorary national extension fraternity. He was recording secretary of the local chapter of the fraternity for several years.

Claude Stever Bryan, Dean, Michigan State College School of Veterinary Medicine, died on July 30, 1951, after a short illness.

Dean Bryan was born June 5, 1908 in Bedminster, Pa. He received his B.S. degree from Pennsylvania State College in 1930, his M.S. Ph.D., and D.V.M. degrees from Michigan State College in 1932, 1937 and 1942, respectively.

Dr. Bryan joined the Michigan State College faculty in 1930 as a graduate assistant. He was successively appointed instructor, assistant professor, professor and head of the Department of Surgery and Medicine and, in 1947, dean of the Veterinary School. An untiring investigator and scientist, Dean Bryan was an authority on bovine mastitis and dairy hygiene. He was an excellent teacher and a great leader. Immediately prior to his untimely death, Dr. Bryan had been engrossed in the building of a new structure, housing the School of Veterinary Medicine, which is now being completed as a result of his vision and effort.

Dr. Bryan was a member of many professional, academic and fraternal societies; among them were the American Dairy Science Association, American Veterinary Medical Association, American Public Health Association, International Association of Milk and Food Sanitarians, Michigan Academy of Science, National Educational Association, Kiwanis International, Sigma Xi, Phi Sigma, and Phi Zeta.

Dean Bryan is survived by his widow, Mrs. Jean Miller Bryan, and two daughters, Marjorie Ann and Nelda Jane.

William L. Clevenger, Raleigh, N. C., died in his sleep in a University of Tennessee dormitory room June 8, 1951. He was in Knoxville attending the 1951 meeting of the American Dairy Science Association.

Professor Clevenger was born October 7, 1881, in Shelby County, Ohio, and had been a member of the faculty of North Carolina State College for about 29 years. He was a graduate of Ohio State University in dairy manufacturing. He was on the staff of Ohio State University for a while following his graduation. He later became associated with the Dairy Division, United States Department of Agriculture, and was assigned to Tennessee and North Carolina to do field work in dairy manufacturing. Later he joined North Carolina State College as Professor of Dairy Manufacturing and did field work with dairy manufacturing plants.

Each of the many dairy manufacturing plants with which Professor Clevenger worked, and which followed his advice, stand today as monuments to the soundness of his leadership.

William M. Crownover was born August 26, 1906, at Antlers, Oklahoma. He graduated from Oklahoma A. & M. College in 1929, receiving his B.S. degree in Dairy Manufacturing. He received the A. C. Baer Memorial award for the outstanding student in dairy manufacturing. Following his graduation he moved to Tulsa, Oklahoma, where he was employed by the Carnation Milk Co. and the Crawford Drug Co. He married Miss Thelma Hasey at Stillwater on July 24, 1930.

In 1937, Mr. Crownover purchased a creamery in Pawhuska, Oklahoma, which was known as the W. M. Crownover Creamery. He operated it until August 20, 1951, when he was advised to sell the business because of ill health. He died on September 8, 1951.

Mr. Crownover's civic activities included the following: Chairman of Osage County Red Cross Organization; past president of the Jr. and Sr. Chambers of Commerce; Chairman of the A. & M. Alumni Organization for Washington and Osage counties; Chairman of the board of First Christian Church. He was a member of the American Dairy Science Association, the Kiwanis Club, Knights of Pythias and IOOF.

He is survived by his wife, a daughter, Rae, and a son, James William, all of Pawhuska.

Charles G. McBride, aged 65, member of the Ohio State University Department of Agricultural Economics and Rural Sociology staff since 1922, died at his home in Columbus on June 10, 1951, after a brief illness.

He would soon have completed 29 years teaching and research service at the University. He was a member of the International Conference of Agricultural Economists and attended conferences of this group in England, Scotland and Canada. He was also a member of the Com-

mittee of Milk Production and Distribution of the National Research Council, The American Dairy Science Association and the Optimist Club of Columbus. For several years he was associated with the organization now known as The Milk Industry Foundation.

Dr. McBride has served the dairy and milk industry of Ohio and the nation well during his 30 years of service. He did considerable work in arbitrating milk market disagreements in former years. He has worked in Kansas City, Oklahoma City, Detroit, New York and many Ohio markets. He worked closely with all of the cooperating milk marketing groups of the state during that period.

From 1933 to 1935, while on leave of absence at Ohio State University, he served as the executive secretary of the Ohio State Milk Commission during the time of the Burke Act. One characteristic of Dr. McBride which meant much to cooperative leaders in the state through the years was his quick appreciation of the position of and his insistence on fair treatment for the milk producer. He was especially interested in training young men for dairy marketing and community work.

He is survived by his widow, Lois, and a son, Harold.

Robert H. Ruffner, a native of Warrenton, Va., died on October 16, 1951, at Rowan County Hospital in Salisbury, N. C., at the age of 69.

Professor Ruffner retired from the North Carolina State College Faculty on June 30, 1950, after more than 30 years on the institution's staff. At one time, he was head of the Department of Animal Industry. He was appointed Professor of Animal Husbandry and Dairying at the State College in 1919 and assumed his duties on October 1 of that year.

Professor Ruffner was born in Warrenton, Va., on May 22, 1882, and was educated at the Rockingham Military Institute, the College of William and Mary, and the University of Maryland. He later did graduate work at Cornell University and North Carolina State College. He was an excellent teacher, greatly beloved by his students, a jovial companion and a sincere and conscientious worker.

Respectfully submitted, Leonard R. Dowd; J. B. Frye, Jr.; Ivan H. Loughary; G. E. Raithby; H. C. Olson, *Chairman.*

Following a minute of silent tribute to the departed, the report was accepted.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

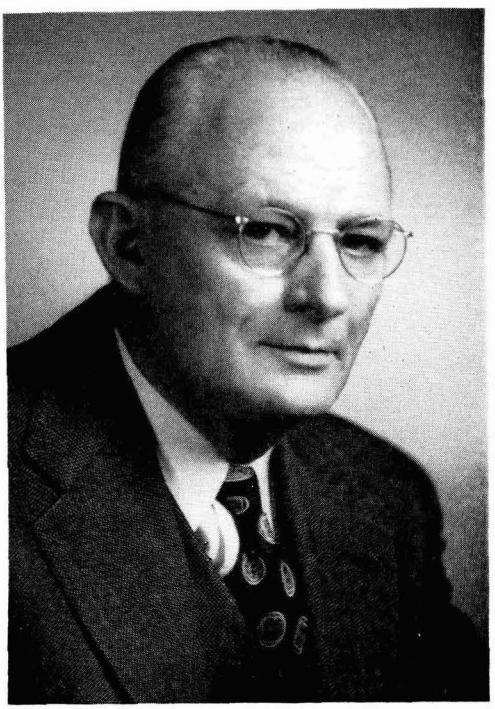
Davis, California, June 25, 1952

The Association Awards presentation took place in the Recreation Hall at 8:30 p.m. Chairman E. L. Jack presented President H. A. Bendixen who presided at the session.

DeLAVAL DAIRY EXTENSION ACHIEVEMENT AWARD

L. H. Rich, member of the DeLaval Award Committee:

"The candidate for the DeLaval Extension Dairyman's Award for 1952 received his academic training at Michigan State College, Pennsylvania State College and Cornell University. He was a leader in the germ plasm survey in 1935, and used the results of this survey for developing a pro-



S. J. BROWNELL

cedure of herd analysis that is known the country over. He promoted and practiced artificial breeding of dairy cattle several years before it was developed on a cooperative basis in Denmark. In fact, he gave a demonstration of the technique at the annual meeting of this Association in 1934. Through his personal example and organization of extension programs he has trained a number of coworkers who have gone into other states and developed outstanding extension programs. He has had two calls by the Federal Government to go to the occupied countries to help plan livestock programs. He has won the respect and confidence of the dairy leaders, and has contributed to many worth-while changes in dairy extension teachings and practices that have

been adopted throughout the United States. He is the author of a number of extension bulletins. But with all these accomplishments for which he is being honored today, he is modest and friendly with everyone.

The man that the committee selected to receive the Award is Professor Stanley J. Brownell of Cornell University, Ithaca, N. Y."

Mr. E. C. Elvidge, Vice President, DeLaval Pacific Co., presented Mr. Brownell with a check for $1000 and an inscribed, framed scroll.

AMERICAN FEED MANUFACTURERS AWARD

T. W. Gullickson, member of the Feed Manufacturers Award Committee:

"For the fifth successive year it has been the privilege of the American Dairy Science Association, represented by a designated committee, to select a worthy candidate for the American Feed Manufacturers Association Award, which is granted for the purpose of recognizing outstanding contributions in dairy cattle nutrition and for stimulating constructive research in this field.

H. D. Eaton

"Though many noteworthy studies were reported during 1950 and 1951, evaluation of the various contributions, on the basis of standards established by the American Dairy Science Association, led to the selection of a comprehensive and progressive series of investigations on the Vitamin A nutrition of young dairy cattle. Fundamentally, the findings have increased information on the utilization of vitamin A and carotene by the developing calf, prenatally and postnatally, and have added to the criteria that may be used in delineating vitamin A deficiencies and in predicting the degree of depletion. Practically, the results from the studies have augmented knowledge of the relation of various methods of processing forages to their value as a source of vitamin A activity for calves.

"The leader in these studies was born in New York. He received his undergraduate training at Johns Hopkins University, at Stanford University and finally at Iowa State College, where he was awarded a B.S. degree in Dairy Husbandry in 1939. He completed work for his M.S. degree in Dairy Husbandry at Rutgers University in 1941 and his Ph.D. degree in Animal Husbandry at Cornell University in 1947. During his graduate career he assisted in teaching and research. Since 1947, he has been a member of the Animal Industry staff of the University of Connecticut, serving initially as Assistant Professor of Animal Nutrition and currently as Associate Professor. Throughout his professional career his zeal for research has been reflected in energetic explorations and fruitful results.

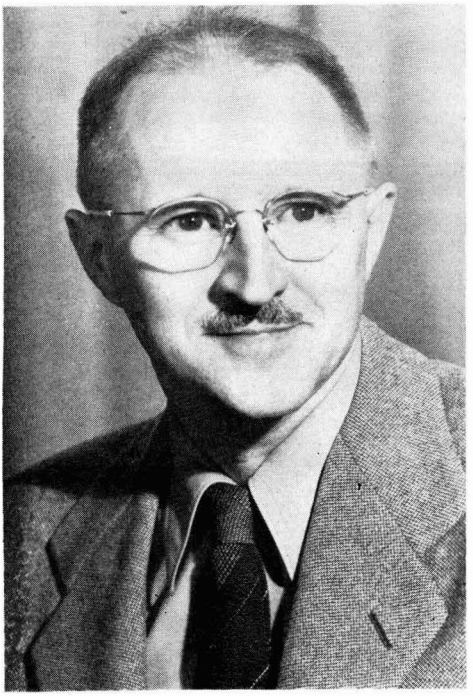
"On behalf of the American Feed Manufacturers Award Committee of The American Dairy Science Association, it is a pleasure to present Dr. Hamilton E. Eaton as candidate of this Association for the 1952 American Feed Manufacturers Award."

Mr. Wm. T. Diamond, Executive Secretary, American Feed Manufacturers Association then presented Dr. Eaton with a check for $1000.

BORDEN AWARD FOR DAIRY MANUFACTURING RESEARCH

P. F. Sharp, member of the Borden Manufacturing Research Award Committee:

"This year's recipient of the Award has been engaged in teaching and research for 25 years and as a consultant to industry in dairy manufacturing for more than 15 years. During World War II he served as a member of the Advisory Board, Quartermaster Corps, United States Army. Over the span of years he has covered intensively a wide field of fundamental and applied research. His subject matter and publications have concerned themselves principally with the technology and chemistry of butter and buttermaking, acidity in butter fat, buttermilk testing reagents, oxidized flavors in ice cream, and vacreation. In recent years much of his interest has been devoted to studies on the influence of



E. W. BIRD

J. W. HIBBS

feeds upon flavor and milk fat, methods of determining total solids, milk adulteration, detection of vegetable fat in milk fat and the chemistry of milk fat.

"The recipient was born in Philadelphia, Pa., on August 20th, 1901. He received his B.S. degree from The Pennsylvania State College in 1923 and his Ph.D. degree from Iowa State College in 1929. He has been at Iowa State College since 1923, where he has risen from a graduate student in Chemistry to a full professorship in Dairy Industry in 1947."

Mr. J. H. Erb, Vice President in Charge of Production, Mid-West Division, The Borden Co., presented Dr. Bird with a gold medal and a check for $1000.

BORDEN AWARD FOR DAIRY PRODUCTION RESEARCH

P. L. Kelly, Chairman, Borden Production Research Award Committee:

"The man who has been selected to receive the Borden Award in dairy production for 1952 was born in Ohio. He received his B.S., his M.S. and his Ph.D. degrees from Ohio State University. After finishing his graduate work he remained on the staff of that University. He is now at the Wooster Agricultural Experiment Station where he has the title of Associate Professor.

"He is the author or co-author of approximately forty scientific publications in the fields of calf raising, rumen physiology, milk fever and the vitamin A and carotene content of hays. These include a review published in the *Journal of Dairy Science* on milk fever.

"In behalf of the Borden Award Committee for Dairy Production, it gives me great pleasure to ask Dr. John W. Hibbs to come forward to receive the Award for 1952."

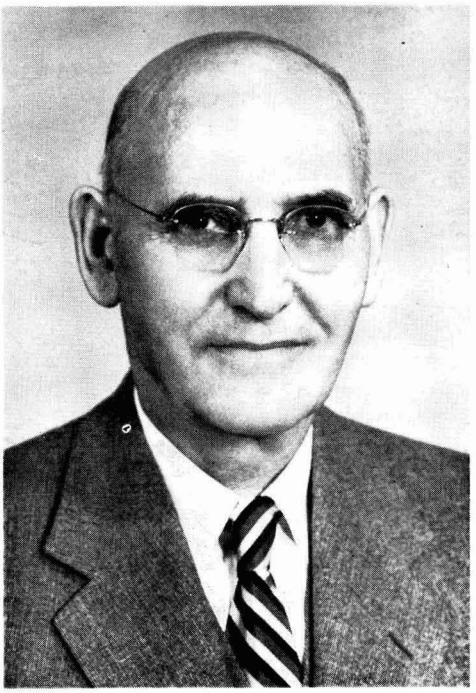
Mr. J. H. Erb, Vice-President in Charge of Production, Mid-West Division, The Borden Co., then presented Dr. Hibbs with a gold medal and a check for $1000.

ASSOCIATION HONORARY MEMBER AWARD

G. M. Trout, member of the Honors Committee:

"Dr. Chester Linwood Roadhouse was born in Watsonville, Calif., January 5, 1881. He obtained the D. V. M. degree at Cornell University in 1906 and entered the service of the U. S. Bureau of Animal Industry. In 1909 he inaugurated the sanitary supervision of San Francisco's milk supply and the following year he was in charge of the milk supply and operation of a certified dairy in Berkeley. In 1911 he joined the faculty of the University of California and

in 1917 went to Davis as head of the Dairy Industry Division. From that time until his retirement he has been actively engaged in research, instruction and administration. Illness in 1945 made it necessary for him to relinquish his administrative duties, but he returned to carry on an active teaching program. In 1951 he retired from the University of California after 40 years of service, but has continued to be active in civic affairs.



C. L. ROADHOUSE

"Dr. Roadhouse has been most active in dairy associations. He was President of the American Dairy Science Association in 1935 and served on the editorial board of the *Journal of Dairy Science* from 1917 to 1926. He was President of the Pacific Slope Dairy Association from 1922 to 1946, inclusive, and President of the International Association of Milk Sanitarians in 1921. He had the distinction of being an official U. S. delegate to the World's Dairy Congress in Berlin in 1937. He spent a sabbatical year at the Swiss Dairy Research Station at Berne, Switzerland. He is an active member of the Institute of Food Technologists, the California Academy of Science, the American Dairy Science Association, Sigma Xi, Alpha Zeta and Theta Delta Chi.

"Some 101 publications appear in scientific literature under Dr. Roadhouse's authorship or co-authorship. These papers deal with the sanitation, flavors and chemistry of milk. In collaboration with Dr. J. L. Henderson, he wrote and recently revised a widely used text on "The Market-Milk Industry."

"Dr. Roadhouse's role as a teacher and counselor is particularly noteworthy. His students are leaders in California dairy industry and several have achieved national recognition.

"He has always been a strong supporter of civic improvement and has contributed in many ways to the growth of his home community. He is a charter member of the Davis Rotary Club and has served on innumerable civic committees. His latest contribution has been to serve as President of the Davis Chamber of Commerce during 1951."

INSTALLATION OF ASSOCIATION OFFICERS

President Bendixen installed the following officers-elect:

"Mr. H. B. Henderson you are about to take over the responsibilities of President of the American Dairy Science Association. As President it will be your duty to preside over the Executive Board and submit to the Board for approval nominations of members to fill vacancies that may occur among the elected officers of the Association. As president you shall appoint the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now confer upon you the honor of being President of the American Dairy Science Association.

"W. V. Price, you are about to take over the responsibilities of Vice-President of the American Dairy Science Association. As Vice-President it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Henderson's term, you will automatically become President of this Association. I now charge you with these duties.

"N. N. Allen and L. H. Rich, you were elected to the Board of Directors of the American Dairy Science Association. It will be your duty to pass on all applications for the establishment of divisions, sections and student branches of the Association. With the other members of the Board you will have full control of the budget and general business of the Association, hold title to all property and funds of the Association and have all the rights and powers vested in the Association by the laws of the District of Columbia. With these privileges, responsibilities and obligations you are now members of the Executive Board of the American Dairy Science Association to serve a term of 3 yr."

NOTICE TO CONTRIBUTORS

Authorship of Original Articles and Reviews.—Space in the JOURNAL is reserved for the publication of original research voluntarily submitted by members of the Association to the JOURNAL and review articles by invitation. In the case of joint authorship, the membership ruling applies to one author only.

Papers that already have appeared in print or that are intended for simultaneous publication elsewhere will not be accepted.

Manuscripts.—Manuscripts should be submitted in double spacing on one side of suitable $8\frac{1}{2}'' \times 11''$ paper. The original copy should be furnished. All illustrative and tabular material should accompany the manuscript.

Except in cases of invited reviews, papers must be limited to 12 printed pages unless previous permission from the editor is obtained. When non-review articles exceed 12 pages, a charge of $5 per over page is made.

Manuscripts will be published in the order of their receipt. They should be sent to the Editor, F. E. Nelson, Dept. of Dairy Industry, Iowa State College, Ames, Iowa. In order to speed publication, one author should be designated to assume the responsibility of checking the galley on all papers of multiple authorship. All galleys should be returned in the minimum possible time to avoid delay in publication.

Figures.—Original drawings, diagrams and charts should be done in India ink on tracing cloth (or white board) not larger than standard letter size ($8\frac{1}{2}'' \times 11''$). All lettering should be inked in block style and be of such size that the lettering will be not less than $\frac{1}{8}$ in. in height when the figure is reduced to 4 in. in maximum dimension. Typewritten labeling of axes and axis units is not acceptable. Original drawings should be submitted, rather than photographs of such drawings. When suitable drawings are not furnished, the author will be charged for the preparation of drawings of satisfactory quality by an independent agency.

Photographs.—Photographs for halftone reproduction should be glossy prints free of all imperfections.

Legends.—Legends for figures and photographs should be typed on a sheet separate from the illustrative material and should be made as concise as possible while retaining their descriptive character.

Tabular Material.—Tabular material should be clear, concise and accurate. Often data can be condensed and presented in summarized tabular form. Tables of only one or two lines should be avoided except in most unusual cases. Excessively large or complicated tables are almost impossible to print satisfactorily. Headings should be as concise as possible, yet descriptive in character. Data may be presented in either tabular form or in figures, but the same data must not be presented in both forms. Each table should be placed on a separate sheet and not in the body of the manuscript. The letters *a*, *b*, *c*, etc., should be used for footnote designations. If possible, tables should be so organized that they may be set across the page, rather than the length of the page.

References.—Literature reviews should be limited to only the most pertinent references. Reference lists should be double spaced and arranged alphabetically as to author and by chronological appearance of the journals cited under a given author. Papers by a single author always precede papers by that author and associates. References to multiple authors are arranged in the alphabetical order of the several authors. Give only initials rather than full first names of male authors. Citations in the text should be made by the number in parentheses, corresponding to the number in the reference list.

Each reference should contain the following: Reference number, author(s), title of article, name of journal, volume number, first and last page numbers, and year of publication. Titles of all articles should appear in complete untranslated form. Consult recent published articles in the JOURNAL for proper citation. Publications are abbreviated according to the form given in CHEMICAL ABSTRACTS, vol. 40, no. 24, part 2. 1946.

Sample of journal citation: (1) JONES, L. W., AND SMITH, J. D. Effect of Feed on Body of Butter. J. DAIRY SCI., 24: 550–560. 1941.

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

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

The more common abbreviations used in the text are: cm., centimeter(s); cc., cubic centimeter(s); g., gram(s); mg., milligram(s); γ, microgram(s); ml., milliliter(s); mμ, millimicron(s); C., Centrigrade; F., Fahrenheit; lb., pound(s); oz., ounce(s).

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In preparing manuscripts, use of first person should be avoided.