JOURNAL OF DAIRY SCIENCE

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THE EFFECT OF ANTIBIOTICS ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS[1, 2]

R. H. WASSERMAN,[3, 4] C. W. DUNCAN, E. S. CHURCHILL AND C. F. HUFFMAN

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Antibiotics and other bacteriostatic agents have been used in isolation work for many years to inhibit selectively undesirable types of organisms (8, 19, 25). Another important use of some of the bacteriostatic agents has been in the identification of certain bacteria which grow in the presence or absence of these agents. The use of antibiotics in rumen digestion studies suggests the possibility of defining the cellulolytic fraction of the rumen microflora in terms of resistance or sensitivity to these agents.

The factors responsible for cellulolytic digestion have been attributed to the naturally occurring microflora in the rumen. Henneberg (12) and Baker and Harriss (1) studied microscopically the partially digested material of the rumen and observed cellulolytic bacteria within the eroded cavities of plant material. Gall *et al.* (9) and Hungate (14) isolated many types of cellulolytic microorganisms in numbers high enough to designate them as major physiologic types.

The *in vitro* method of studying rumen digestion allows greater control and more extensive observations under experimental conditions than does the *in vivo* method, but it is apparent that conditions set up artificially must be substantially equal to the environmental conditions in the intact rumen. Marston (20) devised a method for regulating the pH, maintaining the temperature at 39° C., and maintaining anaerobiosis by passing nitrogen through the system continuously. Louw *et al.* (17) introduced a method for removing the lower fatty acids formed during digestion by suspending a semipermeable sac in a large volume of growth medium, so the acids could dialyze out of the field of fermentation. The results obtained by Louw *et al.* showed that the rate of digestion of cellulose was more rapid in the semipermeable sac than in an all-glass fermentation vat. More recently, Gall and Glaws (10) studied the bacteriology of both the semipermeable

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[2] This study was made in part with funds provided by the Research and Marketing Act of 1946, through a cooperative project between the Michigan Agricultural Experiment Station and the Bureau of Dairy Industry.

[3] Agent of the Bureau of Dairy Industry, U.S.D.A.

[4] This article is part of a dissertation presented to the Faculty of the Graduate School of Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

system and the all-glass, impermeable system and found that the gram stains and culturability of the microorganisms obtained from the semipermeable system compared favorably with those found in the original rumen contents. The impermeable system gave poor culturability, altered gram stains and evidenced a lack of anaerobic organisms.

Using modifications of the above techniques in the operation of an artificial rumen, incubation studies have been made to ascertain the persistency and effect of various antibiotics on rumen microflora and to obtain also information on the cellulolytic activity of the microflora in the presence of antibiotics.

EXPERIMENTAL

Modifications of the methods of Louw *et al.* (17) and Marston (20) were utilized in making an artificial rumen set-up. A semipermeable sac (Visking sausage casing) was suspended in a 2-l. large-mouth glass bottle which contained a complex salt solution somewhat similar to that employed by Burroughs *et al.* (4) and Louw *et al.* (17). The salt solution contained 5.50 g. $NaH_2PO_4 \cdot H_2O$, 3.35 g. $NaHCO_3$, 5.00 g. $MgSO_4 \cdot 7H_2O$, 1.50 g. KCl, 5.25 g. $(NH_4)_2SO_4$, 1.45 g. $CaCl_2 \cdot 2H_2O$, 4.0 mg. $FeSO_4 \cdot 7H_2O$, 4.0 mg. $CoCl_2 \cdot 6H_2O$, 4.0 mg. $ZnSO_4 \cdot 7H_2O$ and 2.0 mg. $CuSO_4 \cdot 5H_2O$ in 2,000 ml. of distilled water. The solution was sterilized in 2-l. quantities by autoclaving for 30 min. at 15 lb. pressure.

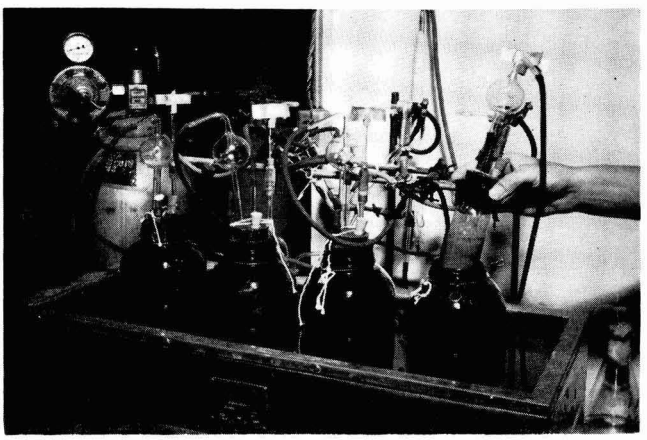

FIG. 1. A battery of artificial rumens assembled for a digestion trial.

The semipermeable sac was 1.75 in. in diameter and closed at the lower end with a no. 10 solid rubber stopper. The top of the sac was closed with a no. 10 rubber stopper which contained two small holes for the gas inlet and outlet tubes and a larger hole, fitted with a no. 00 solid rubber stopper, through which the rumen ingesta and antibiotics were added and samples removed for analysis. The upper no. 10 stopper was fitted into a no. 12 rubber stopper which fitted the mouth of the bottle. The no. 12 stopper also contained two additional holes, one for a mercury-sealed, air-driven stirrer and one for a hypodermic needle. Figure 1 shows a battery of four artificial rumens assembled for a digestion trial.

Anaerobic conditions were maintained in the fermentation sac by bubbling oxygen-free CO_2 through the system continuously. After passing the CO_2 through a chromous acid solution to remove traces of O_2, the CO_2 was washed in distilled water, dried over anhydrous $CaCl_2$, sterilized by passage through an 18-in. column of sterile cotton and then led into the fermentation system. The gas outlet consisted of a Kjeldahl connecting bulb which provided a trap for the liquid and foam that were forced up when the gas was flowing at a high rate. When the flow was decreased, the trapped liquid ran back into the fermentation sac. To expedite the dialysis of the by-products of cellulose digestion, the salt solution outside of the sac was kept in constant motion by use of a stirrer.

To provide the substrate at about the 2 per cent level, approximately 4.0 g. of accurately weighed, finely ground Whatman no. 12 filter paper were placed in each sac. The total amount of cellulose in each sac was the added filter paper plus that introduced with the inoculum. Each sac was suspended in a bottle and the entire apparatus was assembled and sterilized by autoclaving for 15 min. at 15 lb. pressure.

The rumen sample was collected in a sterile 1-l. Erlenmeyer flask fitted with a 3-hole rubber stopper which held a large funnel, a gas inlet and a gas outlet tube. The apparatus was insulated by several layers of brown paper. The liquid rumen ingesta were obtained during the height of digestion from a fistulated Holstein cow maintained on a normal hay and grain ration. The fistula cap was removed and the rubber gloves, worn while sampling, were "washed" with rumen ingesta to remove some of the contaminating microorganisms acquired when the cap was removed. A pocket was dug in the rumen contents to facilitate the removal of the more liquid portions. The sample was removed from the pocket with a sterile cup and filtered through six layers of sterile cheese cloth. The semi-solid portion was squeezed manually to remove the liquid in close contact with the partially disintegrated hay. Throughout the collection period, oxygen-free CO_2 was bubbled through the flask to maintain as near to anaerobic conditions as possible. The flask was completely filled with rumen fluid and immediately taken to the laboratory.

Two hundred-ml. aliquots of the rumen liquid were pipetted into each of the sacs containing the cellulose. The fermentation vessels were placed in a water bath with the temperature thermostatically controlled at $40 \pm 1°$ C. The salt solution was added to each bottle until the aqueous levels were the same on both sides of the sac. Equivalent quantities of the antibiotic then were added to the sac and to the salt solution so the concentration was the same on both sides of the membrane. The entire contents within the sac were mixed vigorously by bubbling CO_2 through the mixture for 5 min. and then the rate of flow was decreased to impart a slow movement. The stirrers were started to keep the salt solution in constant motion. Fermentation was terminated at the end of 24 hr. by adding 10 ml. of a 1:10 solution of Roccal and storing the ingesta under refrigeration. The concentration of the antibiotic and the pH were checked periodically and the acids were neutralized with 1 N Na_2CO_3 to maintain the pH between 6.2 and 6.8.

The antibiotics employed were penicillin-G, crystalline sodium salt, lot no. 06298-F, Parke, Davis & Co.; streptomycin, $CaCl_2$ complex, lot no. 2147, Merck & Co., Inc.; neomycin sulfate, research no. 9273-4, The Upjohn Co.; and chloromycetin (synthetic), lot no. 134007, Parke, Davis & Co.

The antibiotics were assayed by the expedient method of Loo *et al.* (15), except that paper discs were used as reservoirs instead of the usual porcelain cylinders. Bacto-mycin assay agar was used for neomycin, streptomycin and chloromycetin. The pH of the agar was adjusted to 7.0 for chloromycetin. Penn-assay base agar and Penn-assay seed agar were used for penicillin. The test organism was *B. subtilis* (A.T.T.C. 3R8788). A spore suspension with a viable count of 27,000,000 per milliliter was used as the inoculum. Standard curves of each antibiotic were obtained by dissolving a weighed amount of the dry antibiotic in a known volume of phosphate buffer, adjusted to pH 7.9 for neomycin and streptomycin, pH 6.0 for penicillin and pH 7.0 for chloromycetin. The stock solutions were diluted to give concentrations equal to the highest concentration employed in the fermentation sac and then serially diluted to five gradient concentrations. Standard curves were based on six replicas. The antibiotic in the fermentation ingesta was determined every 4 to 5 hr. by the same procedure. One-half ml. of the thoroughly mixed ingesta was removed and seeded on the assay plate with filter paper discs. One disc on each plate was saturated with the antibiotic standard to check against variations in the time of incubation, media, etc. Each antibiotic assay was done in triplicate.

The method of Crampton and Maynard (7) was used for the determination of cellulose. The amount of cellulose was determined in a 200-ml. aliquot of rumen ingesta which had not been incubated and this value was added to the weighed amounts of cellulose to obtain the total amount in each sac.

RESULTS

The antibiotics in the aqueous phase of the rumen ingesta were measured periodically to obtain some indication of the rate of disappearance during the fermentation. The zone of inhibition of each assay was evaluated in terms of an antibiotic unit by comparison with the standard curve. The antibiotic persistency curves were obtained by plotting the effective concentration against incubation time. Figure 2 shows that all concentrations of penicillin dropped rapidly to less than 25 per cent of the original value within 45 min. after it had been added and then persisted at a constant level throughout the duration of the fermentation.

Figure 3 shows that all concentrations of streptomycin increased markedly above the initial level within the first 45 min. and persisted for approximately 14 hr. before the concentrations began to decline. At the end of 24 hr., the concentrations were only slightly lower than the initial levels. The top dilution of streptomycin used to determine the standard curve was only 50.5γ, because higher experimental values were not anticipated. Estimated values, therefore, were used to locate the first three points on the curve presented for the 50γ concentration. These points were obtained by extending the slope of the standard curve to determine the estimated concentration at the three assay periods. A syn-

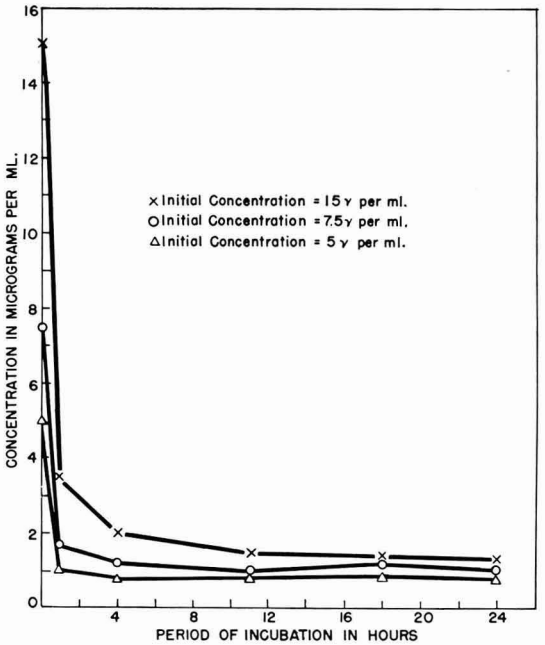

FIG. 2. The *in vitro* rate of disappearance of penicillin in the aqueous phase of rumen ingesta.

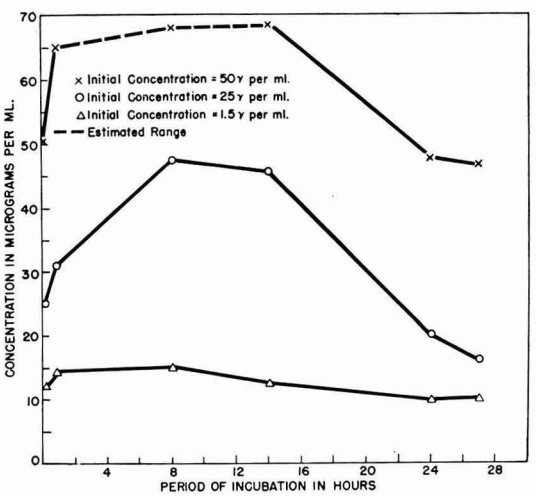

FIG. 3. The *in vitro* rate of disappearance of streptomycin in the aqueous phase of rumen ingesta.

ergistic action with some other constituent in the rumen ingesta may have been responsible for the apparent increase in streptomycin concentration.

The concentrations of neomycin dropped to approximately 60 per cent of the original values during the first 45 min. and then remained fairly constant for the next 7 hr. in the presence of the rumen ingesta. After this time a gradual disappearance was evident, but the aqueous phase still contained 50 per cent of the original amounts after 24 hr. of incubation (fig. 4). Chloromycetin, how-

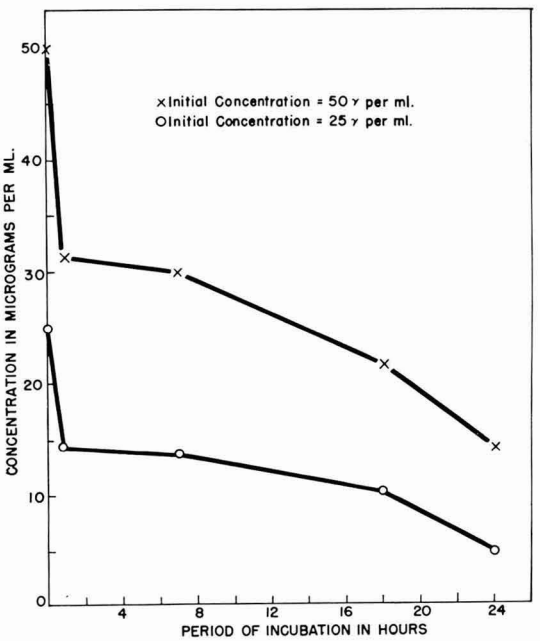

FIG. 4. The *in vitro* rate of disappearance of neomycin in the aqueous phase of rumen ingesta.

ever, disappeared rapidly from the aqueous rumen ingesta. One-third of the antibiotic disappeared within 30 min. in the sac containing 50γ per milliliter, whereas, 60 per cent disappeared in the same time from the sac containing 25γ per milliliter. The 6-hr. assay failed to detect any antibiotic in the aqueous phase of the rumen ingesta. The broken line in this figure was included to indicate that the concentration of chloromycetin was less than 9γ.

Table 1 shows the influence of the various antibiotics on the cellulolytic fraction of the associative population. The lower concentrations of penicillin (5 and 7.5 units per milliliter) stimulated cellulose digestion, but 15 units inhibited digestion. All of the concentrations of neomycin employed in this work increased cellulose digestion, but the stimulatory effects were inversely proportional to the concentrations. Streptomycin did not influence cellulolytic activity significantly when the concentration was 12.5γ per milliliter, but activity was decreased mark-

TABLE 1

The effect of antibiotics on in vitro cellulose digestion

Antibiotics	Concentration	Cellulose			Deviation from control
		Total	Digested	Digested[a]	
	(*units/ml.*)	(*g.*)	(*g.*)	(*per g.*)	(%)
Penicillin	15.0	5.25	1.28	0.244	−35.4
	7.5	5.36	2.41	0.450	+19.0
	5.0	5.10	2.25	0.441	+16.7
	0	5.26	1.99	0.378	.....
	γ/ml.				
Streptomycin	50.0	3.89	1.04	0.267	−13.0
	25.0	3.84	0.92	0.240	−21.8
	12.5	3.84	1.23	0.320	+ 4.2
	0	3.84	1.18	0.307	.....
Neomycin	25.0	4.43	1.30	0.293	+12.3
	12.5	4.33	1.32	0.305	+16.9
	6.25	4.18	1.42	0.340	+30.3
	0	4.63	1.21	0.261	.....
Chloromycetin	50.0	4.32	0.66	0.153	−54.1
	25.0	4.32	1.01	0.234	−29.7
	0	4.27	1.42	0.333	.....

[a] Amount of cellulose digested/gram of total cellulose.

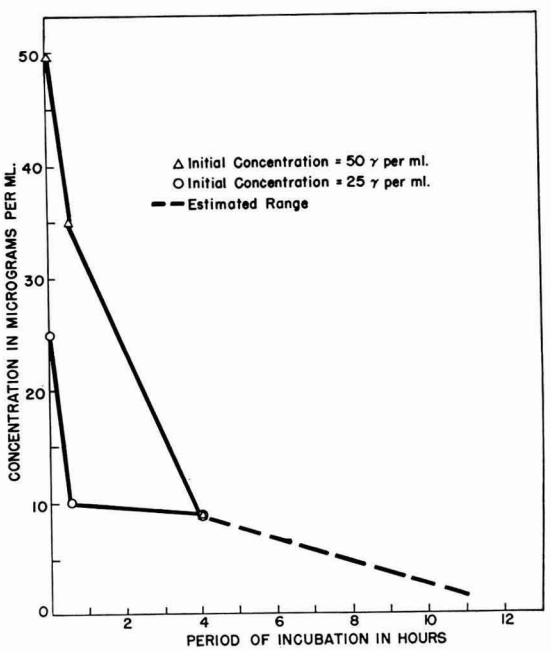

FIG. 5. The *in vitro* rate of disappearance of chloromycetin in the aqueous phase of rumen ingesta.

edly at the higher concentrations. Chloromycetin, in the concentrations employed, markedly inhibited the cellulolytic activity of the rumen microorganisms.

DISCUSSION

Periodic antibiotic assays were made over a 24-hr. period on a heterogenous mixture of cellulose, aqueous rumen contents and various antibiotics to determine the effective concentrations of the antibiotics in the aqueous phase. The filter paper disc method was found to be sufficiently sensitive to determine the concentrations throughout the fermentation period for penicillin, streptomycin and neomycin, but chloromycetin could not be detected at the 6-hr. period. From this, it can only be assumed that the concentration of chloromycetin was less than 9γ per milliliter at the 6-hr. assay.

Bliss and Todd (3) compared eight antibiotics in regard to minimal inhibitory concentrations for gram positive cocci and gram negative bacilli and found that 4 units of penicillin per milliliter inhibited the most resistant microorganisms. Chloromycetin inhibited gram positive cocci and gram negative bacilli in concentrations from 1.2 to 10 and 1.25 to 25γ per milliliter, respectively. With streptomycin, a microorganism is considered sensitive if it is inhibited by concentrations of 10γ or less per milliliter (22). In view of these observations, the cellulolytic fraction of rumen microorganisms appears to be comparatively resistant to penicillin and neomycin, moderately sensitive to streptomycin and sensitive to chloromycetin.

Cohen (6) reported that streptomycin is adsorbed on cellulose. Henry and Hobby (13) suggested that the adsorption of this antibiotic by cellulose would affect the activity of the drug only to the degree of the reduction of free streptomycin in the medium. From the results obtained in this investigation, it is assumed that any adsorbed streptomycin would still be available for bacteriostatic or bactericidal activity, since the drug would be adsorbed on the cellulose substrate of the cellulolytic bacteria. The adsorption phenomenon probably applies also to other antibiotics. The adsorbed antibiotics could not be estimated by the assay method employed, but the results indicate the relative concentrations in the aqueous phase.

Recent studies with growing mice, chicks, young pigs and calves have shown that certain antibiotics increase the rate of growth of these animals (24, 21, 11, 18, 16, 23). Bell *et al.* (2) reported, however, that aureomycin decreased the digestibility of crude fiber in steers on a balance trial involving urea. The observed changes are undoubtedly the result of a change in the microbial population inhabiting the intestinal tract. The present *in vitro* study was employed to indicate possible stimulatory antibiotics. The data suggest that the lower concentrations of penicillin and streptomycin and all concentrations of neomycin appeared to be stimulatory, whereas higher concentrations of streptomycin and chloromycetin inhibited cellulolytic activity. Further work is in progress to study the physiological processes when various antibiotics are incorporated in the ration of ruminants (5).

SUMMARY

The effect of penicillin, streptomycin, neomycin and chloromycetin on *in vitro* cellulose digestion was studied.

Periodic antibiotic assays showed that the concentration of streptomycin increased above the initial level during the first 8 hr. and persisted at a high level throughout the 24-hr. period. Approximately 40 per cent of the neomycin disappeared within 45 min. and then the rate of disappearance was slow for the rest of the period. The concentration of penicillin dropped to less than 25 per cent of the original value within 45 min. and then persisted at a constant level for the rest of the period. The level of chloromycetin decreased rapidly and was not detectable after 4 hr.

In the concentrations used, penicillin stimulated the cellulolytic rumen microorganisms at the lower concentrations, neomycin was stimulatory in all concentrations, streptomycin was slightly stimulatory in the lowest concentration and chloromycetin adversely affected the microorganisms.

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RIBOFLAVIN STUDIES WITH CALVES. I. PREPARATION OF A RIBOFLAVIN-DEFICIENT MILK[1,2]

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A desire to investigate riboflavin metabolism in young calves prompted the development of a riboflavin-deficient ration. For similar studies, Wiese *et al.* (10) used a diet of "synthetic" reconstituted milk and Warner and Sutton (8) used a technique of ultra-violet irradiation to destroy the riboflavin in liquid milk, using a 400-w. mercury vapor lamp emitting rays longer than 3000 Å. This technique of ultra-violet photolysis was modified by Brisson and Sutton (2), who obtained approximately 97 per cent riboflavin destruction in batches of 33 to 35 lb. of milk within 4.5 hr. In this latter case, photolysis took place in a photolyzing chamber specially designed to afford maximum surface exposure while increasing the temperature to 60 to 65° C. After investigating an irradiation procedure, the present authors made further studies to find a method by which the riboflavin could be removed more rapidly from large quantities of milk with a minimum change in the flavor or other characteristics. Since 90 per cent of the riboflavin in milk is in the unbound state (5) and since riboflavin is adsorbed by Florisil (3), the possibility of preparing the desired product by chromatographic procedures was investigated.

EXPERIMENTAL

Destruction of riboflavin by ultra-violet irradiation. Three gal. of skimmilk were placed in a pyrex jar 18 in. in diameter with the surface of the milk 9 in. directly below the source of light. The milk was subjected to irradiation from a 600-w., 12.5-cm. quartz mercury vapor lamp[4] while being stirred mechanically.

Under these conditions, the destruction of riboflavin was relatively slow at room temperature (fig. 1), less than 90 per cent destruction occurring during 12 hr. of irradiation. Five hr. of irradiation at 60 to 65° C. reduced the riboflavin to less than 0.15 mg. per liter, as determined microbiologically (6). The milk was heated by hot plate or by two infra-red and two flood lamps. The longer wave lengths from these lamps did not appear to increase materially the rate of riboflavin destruction. The treated milk assumed a dull, chalky white color and a strongly oxidized flavor.

Removal of riboflavin by chromatography. Florisil (1.5 to 2.0 lb. of 60/100 mesh) was used in preparing a column 3 in. in diameter. The rate of flow was regulated to 5 gal. of milk per hour by vacuum. Skimmilk was effectively chro-

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[2] Data presented in this paper are taken from a thesis submitted by E. G. Moody in partial fulfillment of requirements for a Ph.D. degree, Purdue University, Lafayette, 1951.

[3] Present address: Agriculture Department, Arizona State College, Tempe.

[4] Luxor Model, Hanovia Chemical and Manufacturing Co., Newark, N. J.

matographed cold, but whole milk had to be treated immediately after milking, or it required warming to about 45° C. to prevent clogging of the column, presumably by liquefying the fat. The flavor was not noticeably altered, but the color of the skimmilk and of the milk below the cream line of the whole milk was a dull, chalky white. The cream layer appeared to be unaltered.

The Florisil was reclaimed by successively washing with warm water, acetone or ethanol, and finally ashing at 600 to 650° C.

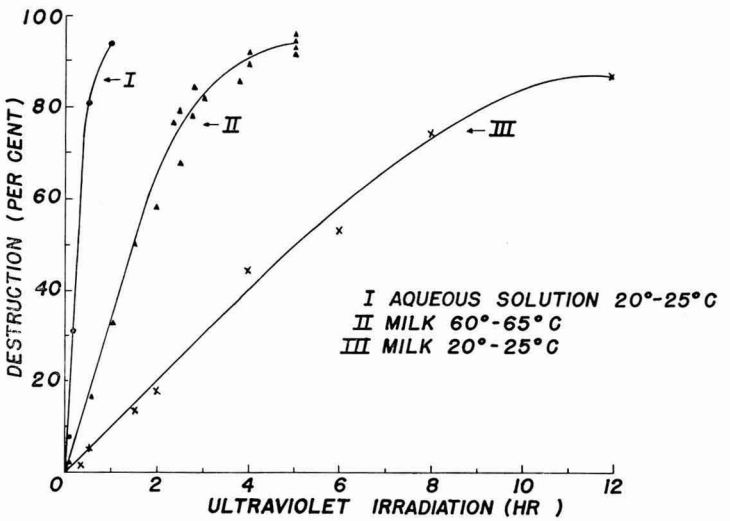

FIG. 1. Destruction by ultra-violet irradiation of riboflavin in an aqueous solution and in milk at 60–65° C. and at 20–25° C.

Riboflavin assay. Untreated and treated milks were assayed for riboflavin by fluorometric procedures (4) using a Coleman model 12 photofluorometer to

TABLE 1

Comparison of riboflavin assay procedures for untreated and treated milk

Treatment of sample	Untreated milk			Chromatographed milk			
	No. samples	Range	Average	No. samples	Range	Average	B_2 removed
		(*mg./l.*)	(*mg./l.*)		(*mg./l.*)	(*mg./l.*)	(%)
Photofluorometric determination:							
67% acetone extract (3)	26	1.45–2.14	1.76	39	0.010–0.090	0.034	98.1
Microbiological determination:							
Direct addition to tubes (5)	12	1.61–3.06	2.54	18	0.143–0.448	0.299	88.5
pH 4.5 filtrate (6)	5	1.26–1.73	1.48	21	0.104–0.299	0.217	85.3
67% acetone extract of hydrolyzed sample	.....	.....	.....	2	0.090–0.128	0.110	.....
67% acetone extract of milk	.....	.....	.....	4	0.020–0.038	0.030	.....
pH 4.5 filtrate (modified standard curve)	.....	.....	.....	3	0.102–0.104	0.120	92.0

measure the fluorescence and/or by microbiological techniques (6, 7). The values as summarized in table 1 represent results obtained from 1949–1951 and do not necessarily represent different assays of the same sample of milk.

Fluorometric and microbiological assay procedures were comparable for untreated milk. However, it was observed that the fluorometric assays gave consistently lower values than those obtained by the microbiological method when applied to the low-potency treated milk. In the microbiological assays where diluted milk was added directly to the tubes (6), high values were obtained. The extraction of some samples with ether (7) usually gave lower values. However, if the milk were hydrolyzed by autoclaving with 0.1 *N* HCl at 15 lb. pressure for 15 min., filtered at pH 4.5 and a neutralized aliquot of the filtrate added to the tubes, lower values were obtained. Comparable results usually were obtained with samples which had been digested with taka-diastase and papain or with 0.1 *N* HCl.

The possibility existed that the discrepancies in the values obtained by the fluorometric and the microbiological determinations of treated milk might be due to differences in efficiency of extraction methods, rather than in the methods of estimation. Samples of hydrolyzed and unhydrolyzed milk were extracted with 67 per cent acetone. After vacuum removal of the acetone, aqueous solutions were assayed microbiologically. The values obtained with the hydrolyzed milk approximated 0.11 mg. per liter, while those with the unhydrolyzed milk were 0.03 mg. per liter; this latter value compared favorably with 0.034, which was obtained with the fluorometric method (4). These results suggested that 67 per cent acetone fails to extract all of the riboflavin from undigested milk. It also indicated that microbiological assay would give similar values to the fluorometric values on the same extract.

To eliminate the effect on the assay results of any possible growth-stimulating substances in the filtrate of acid-hydrolyzed milk, 1 ml. of this filtrate was added to the medium of both the standard and assay tubes. This modification gave values of about 0.12 mg. per liter, as compared with 0.26 per liter when such precaution was not used. The values obtained with this modification were comparable to those obtained by the assay of the acetone extract of the same milk, 0.12 and 0.11 mg. per liter, respectively. Apparently, the effect of unknown stimulants thus was eliminated and these latter values probably more closely approximate the true values of the riboflavin present in chromatographed milk.

Additional vitamins adsorbed. To test the possibility that other vitamins besides riboflavin might be removed from the milk by chromatography, feeding tests were conducted with rats. Weanling rats, 40 to 50 g., were fed a basal ration consisting of 400 g. "vitamin-free" casein, 100 g. hydrogenated cottonseed oil, 1519 g. cerelose, 80 g. minerals, 1 g. cod liver oil and milk *ad libitum*. The vitamin supplements were fed daily in solution in castor cups. Weekly gains were measured. The design of the tests and results are given in table 2.

Trial I indicated that the treated milk was deficient in factors other than riboflavin. Supplementation of the riboflavin-fortified chromatographed milk with thiamine, calcium pantothenate, pyridoxine and choline during the fourth week

gave an immediate growth response, indicating that the chromatographed milk was deficient in at least one of these added vitamins.

TABLE 2

Average weekly gains by rats receiving untreated and chromatographed milk with certain B-vitamin supplements

Lot	Time on experiment (wk.)			
	1	2	3	4[a]
	(*g.*)	(*g.*)	(*g.*)	(*g.*)
Trial I				
1. Untreated milk with no supplements	21.8	31.0	25.8	32.3
2. Treated milk plus riboflavin	22.3	18.8	−4.3	42.3
Trial II				
1. Untreated milk + B-vitamins[b]	22.0	34.8	30.8	29.5
2. Treated milk + B-vitamins	23.3	33.3	34.8	30.3
3. Treated milk + B-vitamins except thiamine	21.3	17.5	0.3	45.0
4. Treated milk + B-vitamins except calcium pantothenate	23.5	31.0	28.8	28.3
5. Treated milk + B-vitamins except pyridoxine	21.0	31.8	26.0	30.8
6. Treated milk + B-vitamins except choline	13.8	23.3	24.3	22.3

[a] Missing factor per lot was supplied the end of the 3rd week.

[b] B-vitamins included the following daily amounts (γ): riboflavin, 40; thiamine, 20; calcium pantothenate, 10; pyridoxine, 9; and choline, 4,000.

Trial II was designed to determine which of the four vitamins had been removed in addition to the riboflavin. The tests indicated that thiamine was definitely deficient and possibly choline. The deficiency of thiamine in the chromatographed milk was confirmed by analysis, using the fluorometric method (1). The thiamine contents of milk samples before and after chromatography were as follows:

Untreated (*mg./l.*)	Chromatographed (*mg./l.*)	Per cent removed
0.405	0.054	86.7
0.473	0.059	87.6
0.347	0.047	86.4

The choline contents of milk before and after chromatography were determined by a modification of the Engel method, (9) which indicated little or no loss of choline during chromatography.

A slight loss of butterfat during chromatography was indicated by Babcock tests which showed that the milk contained 3.9 to 4.2 and 3.7 to 4.0 per cent butterfat, respectively, before and after chromatography.

DISCUSSION

The results presented in fig. 1 show that although the destruction of riboflavin by irradiation with ultra-violet light was fairly rapid in aqueous solution, the destruction in milk was relatively slow at room temperature but much more rapid at higher temperatures. Although the photolyzing process effectively destroyed more than 90 per cent of the riboflavin in milk, the rate of destruction

using these techniques would limit the amount of milk that could be prepared and, consequently, the number of calves that could be maintained on the experimental diet at any one time.

Chromatography offered a possibility of preparing large quantities of milk in a relatively short time. Of the possible adsorbents, Florisil had the advantage of not only adsorbing riboflavin but, because of its white color, it was easy to follow this process on the column. It was found that riboflavin and thiamine probably were the only vitamins removed from milk. There appeared to be no alteration in flavor or consistency of the milk. This indicated that chromatographed milk might be used in the diet to study the riboflavin metabolism of calves.

Furthermore, the process offers a practical and inexpensive means of producing a thiamine-deficient diet for studies of this vitamin. Inasmuch as the flavor of chromatographed milk apparently is unaffected, the technique might have some value also in human nutrition studies.

The apparent discrepancies in the riboflavin values obtained by the photofluorometric method and the usual microbiological assay were disconcerting until it was found that the microbiological assays of the acetone extract of milk gave values comparable to those obtained by the photofluorometric method. This observation indicated that the low fluorometric values obtained were due to incomplete extraction of riboflavin by the acetone. This became evident when acetone extracts of hydrolyzed milk gave higher microbiological values. The results of this experiment indicate that acetone extracts only the free riboflavin and not the bound form, which conclusion is in contradiction to the opinion of Hand (4) who stated that both forms were extracted. Relatively, this 10 per cent of the riboflavin that is bound has a greater effect on assay results of low-potency milk than of untreated milk.

The high values which were obtained by microbiological assay were found to be due to growth stimulants in the milk not removed by usual extracting techniques. When the procedure was modified to minimize the effect of their presence, the values obtained with acid-hydrolyzed milk by the microbiological assay approached those obtained with the acetone extract of the same samples.

Since about 90 per cent of the riboflavin in milk is in the free state, it is likely that chromatography can remove no more than this amount (table 1).

SUMMARY

A low-riboflavin potency milk suitable for experimental feeding was obtained either by photolysis with ultra-violet light or by chromatography with Florisil.

Chromatography removed from normal milk about 90 per cent of the riboflavin and 85 per cent of the thiamine, yielding a product containing about 0.12 and 0.053 mg. per liter, respectively. Other constituents and properties, including flavor, apparently were unaffected by chromatography.

The low-potency milk yielded a value of 0.22 mg. riboflavin per liter when assayed by ordinary microbiological procedures and 0.034 mg. per liter when the acetone extract was assayed photofluorometrically; however, a more precise value

of 0.12 mg. riboflavin per liter was obtained when the modifications described herein were employed.

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THE RELATION OF PREPARTAL AND POSTPARTAL MINERAL BALANCES TO THE OCCURRENCE OF PARTURIENT PARESIS IN DAIRY COWS[1, 2, 3]

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Much information is available in the scientific literature concerning the levels of various mineral elements in the blood of dairy cows at the time of parturition. It also is known that the blood levels of certain of these elements, *e.g.*, Ca, Mg, P, differ greatly from normal in cows afflicted with parturient paresis (milk fever). Knowledge of the mineral levels in the blood of cows with this disorder is interesting, but without supporting information it does little to elucidate the etiology of the disease.

The authors have been unable to find a study of the complete mineral balance picture in a number of cows near the time of parturition. If such information were available, any differences in the prepartal and postpartal balances between normally calving and milk-fever cows could be noted and related to the occurrence of milk fever.

The balance studies of Forbes *et al.* (4) included studies on nine cows near the time of parturition. These workers found that Ca excretion in the urine was lowest in cows recently fresh, and that Cl excretion was highest in dry cows. Na and K balances did not appear to be related.

The urinary excretion studies by Blosser and Smith (1) showed no definite relation between the blood picture for Ca, Mg and P, and the urinary excretion of these constituents. However, Mg excretion via urinary channels was much higher between 16 and 3 days prepartum in cows subsequently developing milk fever than in normally calving cows.

The purpose of the experiment reported herein was to study the complete mineral balance picture on certain minerals near the time of parturition in several cows, some of which were expected to have milk fever.

EXPERIMENTAL METHODS

Sixteen parturitions were studied in 14 cows selected from The State College of Washington dairy herd. The cows in this study were divided into four groups:

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[1] These data were taken from a thesis presented by the senior author to the faculty of The State College of Washington in partial fulfillment of the requirements for the Doctor of Philosophy degree.

[2] Scientific Paper no. 1095, Washington Agricultural Experiment Stations, Pullman. Projects 920 and 1053.

[3] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure no. 171.

[4] Department of Dairy Husbandry.

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[6] Division of Industrial Research.

Group I, mature Jersey cows which calved normally; Group II, mature Jerseys which subsequently developed milk fever; Group III, first-calf Jersey heifers; and Group IV, mature Guernseys and Holsteins. Five parturitions were studied in Group I, three in Group II, four in Group III and four in Group IV. In the latter group, there were two Guernseys and two Holsteins. A schedule was set up to collect blood, urine and feces samples from all cows on the 30th, 15th and 10th days, and for 5 consecutive days prior to calving. Difficulty in predicting the date of parturition resulted in a paucity of information on some animals, particularly the primiparous group (Group III), while more data than intended were collected on other cows. The same materials were collected on the day of calving, for 5 consecutive days thereafter, and on the 10th, 15th and 30th days postpartum.

Blood samples were drawn from the external jugular vein, using heparin as the anticoagulant, at the beginning and end of each collection day. All milk was removed from the udder twice daily and weighed and the calf fed from this milk. All feed was weighed on the day of collection and for 2 days previously, and any feed refused was weighed back.

The total daily excretion of feces was collected in a collection stall modeled after one described by Forbes *et al.* (3). Urine was collected at 2-hr. intervals by stimulation. The feces were weighed at the end of the 24-hr. collection period, mixed thoroughly, a representative sample taken and dried in an electric oven and then ground. The urine was collected under toluene, the volume measured and a representative sample to be used for analysis was acidified with HNO_3 to approximately pH 3.0 to prevent formation of insoluble phosphates and carbonates. Blood, milk feces and feed samples were wet ashed with HNO_3 and $HClO_4$. Blood serum Ca on all samples was determined by the method of Clark and Collip (2). Ca on all other samples was determined by the method of Morris *et al.* (6). Na and K in whole blood were determined with a Beckman Model DU flame photometer. Cl was determined in feces and feed by the Volhard method (8) and blood serum and urinary chlorides by the method of Sendroy (7), as modified by Van Slyke and Hillier. Na and K in the urine were determined with the flame photometer after appropriate dilution with water.

RESULTS

Since the data are too extensive to be presented in tabular form for the individual cows, the average data for the four groups of cows are presented graphically in figures 1 and 2. Ward *et al.* (9) have presented, in a previous publication, detailed data on four of the cows used in this study. Complete feed intake data, blood levels and excretion data also are available for each cow (10). The average blood levels and total daily urinary excretion of K, Na and chlorides for the four groups of cows are presented, primarily for convenience in figure 1. The blood Ca levels near the time of parturition were found to be similar to those reported many times for normally calving and milk-fever cows. Ca excretion in the urine was very low and bore no relation to the blood levels. For these reasons, the Ca data are omitted from figure 1.

Blood studies. Figure 1 shows that the whole-blood K levels were subject to less drastic fluctuations than the levels of the other constituents studied. At 10 days prepartum, all groups had nearly the same level of blood K. Between 5 days prepartum and the day of parturition, blood K showed a general, if not

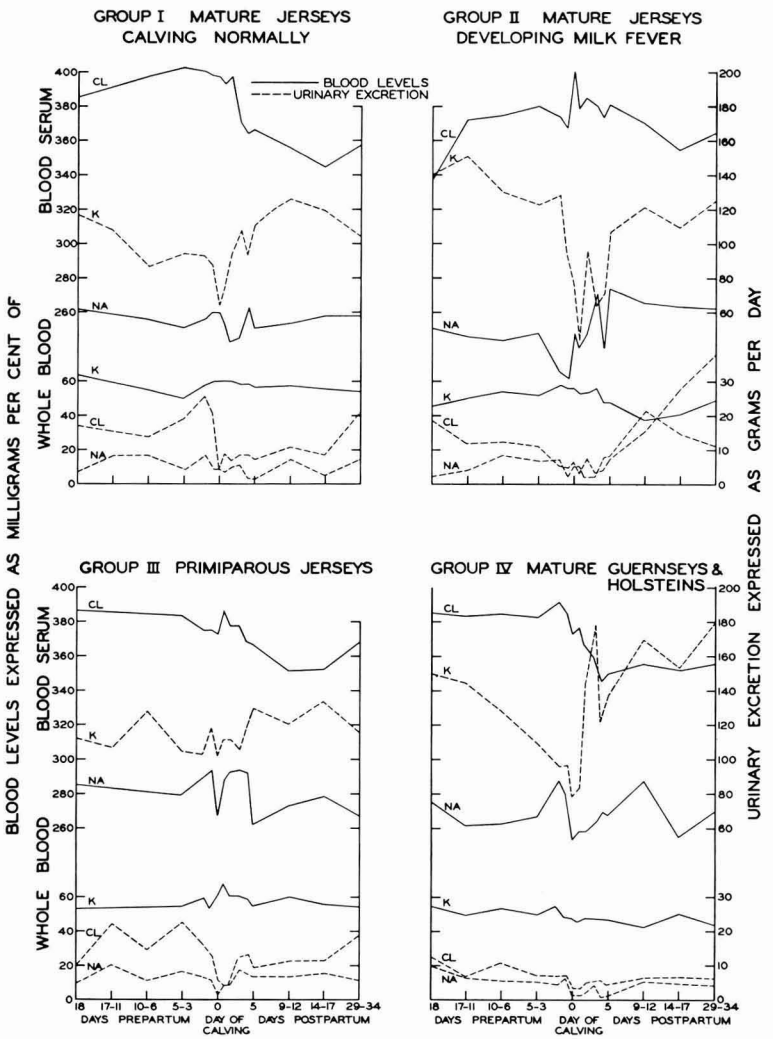

FIG. 1. Blood and urinary levels of Na, K and chlorides for dairy cows at the time of parturition.

very marked increase. The most noticeable postpartal change in blood K was in the milk-fever group, in which the levels dropped markedly between 3 and 9 to 12 days postpartum.

In all four groups of cows, the level of blood Na decreased near the time of parturition. In the mature Jersey cows calving normally, average blood Na levels increased on the day of calving and decreased in the days following. The average blood Na levels for the milk-fever cows were the lowest on the days preceding parturition, while the Na values for the other two groups were lowest on

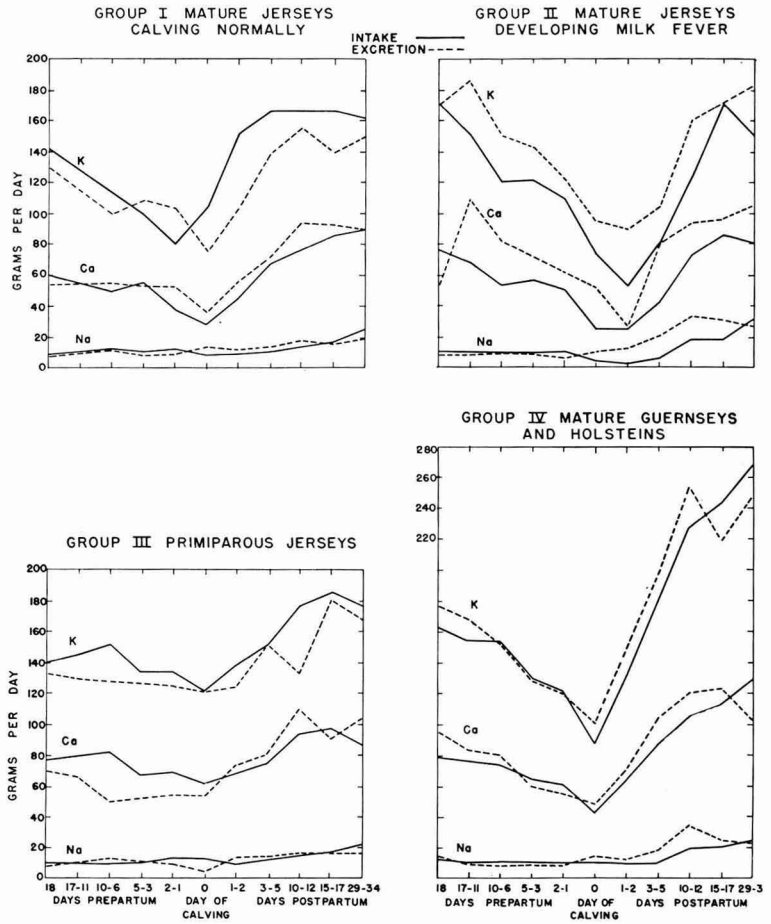

FIG. 2. Intake and excretion of Ca, Na and K for dairy cows at the time of parturition.

the day of calving. The postpartal picture for blood Na in the various groups was very erratic, and, because of the limited amount of data, it is difficult to describe any trend.

There was a general trend toward higher levels of blood-serum chlorides near the day of parturition. With the exception of the cows developing milk fever, blood chloride levels dropped sharply to levels lower than those found prepartum.

Blood chloride levels in the milk-fever cows increased immediately following parturition and were later maintained at levels similar to those found prepartum.

Urinary excretion. The urinary excretion of all constituents decreased at the time of parturition, but the prepartal picture for all groups did not follow exactly the same pattern. The decreased excretion of minerals in the urine at this time can not be explained by a decreased volume of urine, since in most cases an increased volume of urine was voided. In some cases an abnormally large amount of very dilute urine was excreted following parturition. The volume of urine excreted on the day of calving ranged from 3.8 to 22.5 l. The normal urine volume was closely related to the body weight of the cow. K accounts for most of the cation excreted in the urine. The most precipitous drop in the excretion of K occurred in the case of the cows which developed milk fever. In this group the decline as parturition approached was gradual until 2 days prepartum, at which time there was a great drop in K until the day of parturition. There also was a sharp decline in K excretion in the mature Guernseys and Holsteins, but in this group the decline started at 18 days prepartum and continued until the day of parturition. The prepartal decline in urinary K excretion was quite gradual in the mature Jerseys. The first-calf Jerseys were the only group in which marked changes in urinary K excretion did not occur during the period studied. This group did not vary markedly from 120 g. urinary K per day during the entire course of the study.

The postpartal K excretion was similar in all groups except the mature Guernseys and Holsteins. This group showed a tremendous increase in urinary K excretion between the day of parturition and 3 days postpartum (from 80 g. to 180 g. daily). K excretion had declined again by days 4 and 5 postpartum and increased from this time to 9 to 12 days postpartum, at which time excretion of this constituent leveled off at about 170 g. per day.

The urinary excretion of Na and chloride salts was not of the magnitude of K excretion. Urinary Na excretion followed much the same pattern in all groups prepartum. Also it was nearly the same postpartum, except in the group which developed milk fever. In this group, the Na excretion increased considerably between 9 and 12 days postpartum, while all other groups were excreting nearly the same amounts of urinary Na as they had prepartum.

Urinary chloride excretion decreased in all groups as parturition approached. This decline was gradual in the milk-fever and mature Guernseys and Holstein group, but quite abrupt in the first-calf Jerseys and mature Jerseys. In the latter two groups, the urinary chloride excretion was more than double the excretion of chloride in the milk-fever cows or the mature Guernseys and Holsteins between 5 and 2 days prepartum.

The postpartal urinary excretion of chlorides followed much the same pattern in all groups, until 9 to 12 days postpartum. From this point on, the cows which had had milk fever increased much more rapidly in chloride excretion, until 29 to 34 days postpartum, than did cows in the other groups.

Mineral balance picture. This study furnished some of the most interesting information gathered during the course of the experiment. Figure 2 presents

graphically the intake and excretion of K, Na and Ca by the four groups of cows. Similar data for chlorides are not included, because complete data were available on only a few of the cows.

The K balance prepartum was definitely positive in the case of the first-calf Jerseys, borderline in the case of the mature Jerseys calving normally and the mature Guernseys and Holsteins and consistently negative for the milk-fever cows. The postpartal picture showed a definite positive balance in the mature Jerseys calving normally and the first-calf Jerseys, a slight negative balance in the mature Guernseys and Holsteins, and a definite negative balance in the cows which were afflicted with parturient paresis.

Na intake and excretion were about equal except for the cows which developed milk fever, in which case a negative Na balance occurred following calving. To a lesser degree, the mature Guernseys and Holsteins were in negative balance during the same period. Both the intake and excretion of Na were quite low compared with the other constituents studied.

The Ca balance picture prepartum was equally interesting and very similar to the K balances. As was the case with K, Ca balances were definitely and consistently positive in the first-calf Jerseys, borderline in the mature Jerseys calving normally and the mature Guernseys and Holsteins, and definitely negative from 17 to 11 days prepartum on to parturition in the cows which developed milk fever. All cows were in a negative Ca balance following parturition, but this was most pronounced in the milk-fever group.

DISCUSSION

Definite conclusions should be avoided in a study with such a small number of animals in the four groups. As indicated previously, in some cases the prepartal information was meager. Only three parturitions involving milk fever were studied, and two of these cases were in the same cow. In interpreting the data from milk-fever cows, it must be remembered that the treatment with Ca gluconate undoubtedly altered the postpartal data, at least for the first 4 days postpartum.

Blood levels and urinary excretion of Na and K, particularly the latter, are valuable indices of adrenal dysfunction in man and laboratory animals. An increased Na and decreased K excretion generally indicates decreased adrenal activity. The drastic physiological and endocrinological changes which occur at parturition could be expected to alter adrenal efficiency. However, the data presented will not support such a hypothesis, for both Na and K excretion decreased in the days near parturition. If changes in excretion of these cations indicative of adrenal inefficiency occur, the changes must be more transitory than could be detected by the experimental procedure followed in this study.

An increased K and decreased Na level of the blood is a less accurate indication of adrenal insufficiency. Figure 1 shows a decreased blood Na level at or near calving for all groups, while blood K levels increase slightly except in the case of the mature Guernseys and Holsteins. This trend is somewhat more marked in the case of cows which developed milk fever. However, the trends are neither definite enough nor involve enough cows to warrant further assumptions.

Greater differences between groups are apparent in mineral balances presented in figure 2. By far the most interesting and probably the most significant is the difference in Ca balance prepartum. The cows which ultimately came down with milk fever had a severe negative Ca balance for about 15 days prepartum, while the others maintained their Ca reserves, and the primiparous Jerseys appeared to be storing Ca. All cows were in a negative balance following parturition, as has been shown to be the case for all cows studied (2).

The prepartal differences are important, for it is here that changes would be expected to occur which predispose some cows to attacks of parturient paresis. The strongly positive Ca balance for primiparous Jerseys is of great interest, because there never has been a documented report of a cow developing parturient paresis at the time of the first parturition.

If this is a true picture of the prepartal Ca metabolism of cows, it may well be an important factor in the etiology of parturient paresis. The consistent negative Ca balance would result in a depletion of the body reserves. The loss of Ca in the milk following calving, even though the absolute loss were no greater than in normal cows, would represent a proportionately greater loss of the body reserves at the time of calving. It may be that milk-fever attacks are preceded by a period of defective Ca absorption from the gut, or on the other hand it may be the result of excessive Ca excretion into the intestine. The beneficial results obtained with large doses of vitamin D by Hibbs and Pounden (5) might be explained by this phenomenon, for the principal action of vitamin D is to promote absorption or retention of Ca.

No explanation occurs to the authors to account for the severe negative K balance found in the case of the cows which developed milk fever.

Na intake and excretion were about equal, except for the milk-fever groups in the period following parturition, when excretion was considerably higher than the intake.

SUMMARY

Data are presented for average blood and urinary levels of Na, K and chloride on 16 parturitions in dairy cattle over a period from 30 days prepartum to 30 days postpartum. Parturitions were studied in mature Jerseys cows, some of which developed milk fever, in primiparous Jerseys and in mature Guernseys and Holsteins, none of which developed milk fever. The total intake and excretion during this period of Na, K and Ca also is presented for the same animals.

Blood chloride levels were lower, particularly in the period between 5 and 30 days prepartum, in the milk-fever group than in any other group. Postpartal blood chloride levels were as high as prepartal levels only in the milk fever group.

Urinary K excretion in the milk-fever group decreased markedly between 2 days prepartum and the day of parturition. This abrupt decrease in K excretion was not characteristic of any other group.

Urinary chloride excretion decreased more rapidly (and from a higher level) between 5 and 2 days prepartum in the milk-fever group and in the mature Guernseys and Holsteins than in the other groups.

The cows developing milk fever had a severe negative balance of Ca prior to parturition, while the normally calving cows were in positive Ca balance during this period. All cows were in negative Ca balance following parturition.

Cows developing milk fever were in a severe negative K balance throughout the study, while the other groups were nearly in equilibrium.

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THE USE OF METHOXYL GROUPS IN FORAGE AND FECAL MATERIALS AS AN INDEX OF THE FEEDING VALUE OF FORAGES[1]

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Lignin has been used as an indicator of the digestibility and feeding value of forages by many investigators. At the present time the most reliable method of analysis for lignin appears to be the 72 per cent H_2SO_4 method proposed by Ellis *et al.* (5). This method has been developed to isolate an indigestible residue rather than to determine a definite chemical compound.

The application of all methods which have been proposed for the measurement of lignin is laborious. Most procedures require many different treatments to remove interfering substances such as sugars and proteins, temperature control is needed for the enzymatic and acid digestions and the results are not easily reproduced.

The methoxyl content of lignin and the percentage of the total methoxyl in the plant increase with advancing age of the plant (9, 10). The determination of the methoxyl content of plants by the Zeisel method, as outlined by the U. S. Forest Products Laboratory (2), is executed more quickly and easily than the determination of lignin. Since methoxyl appears to be closely associated with lignin and with changes in the plant associated with advancing age, it was indicated that, should methoxyl be related to the composition and digestibility of plants, it might provide an index of the feeding value of forage materials which is as reliable as that provided by lignin.

For these reasons this study was made to investigate the feasibility of using the methoxyl content of forages and/or feces as an index of the digestibility and feeding value of forages.

EXPERIMENTAL PROCEDURE

Pasture forage (predominantly timothy) was studied in hand-feeding and grazing trials during the vegetative, boot-to-early head and full-bloom stages of growth. One group of steers was fed definite quantities of freshly clipped forage, while the other group grazed forage of the same source. The feces of both groups were collected quantitatively in fecal collection bags.

Methoxyl was determined by a modification of the Zeisel method, as outlined by the U. S. Forest Products Laboratory (2). The method proposed by Ellis *et al.* (5) and modified by Thacker (13) was employed for the measurement of lignin. The proximate constituents were determined by the methods suggested by the Association of Official Agricultural Chemists (1).

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The digestibility of the hand-fed forage was determined in conventional digestion trials, while that of forage selected by the grazing steers was determined according to the method proposed by Reid *et al.* (11). The relationships of the methoxyl levels of forages and of feces to the quantities and digestibility of the proximate constituents were examined. Particular attention was given to the relationship between fecal methoxyl and the digestibility of forage dry matter.

RESULTS AND DISCUSSION

The composition of the herbage hand-fed to steers during these trials is shown in table 1. The content of methoxyl and of lignin increased progressively with

TABLE 1

Mean composition and digestibility of forages fed (dry basis)

Growth stage	Methoxyl	Lignin	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Digestible dry matter	
								Hand-fed	Grazed
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Vegetative	1.58	5.8	7.5	16.1	38.1	33.5	4.8	72.9 ± 0.29[a]	77.6 ± 0.47
Boot-to-early-head	1.75	6.7	6.9	12.0	38.3	38.5	4.3	66.3 ± 0.78	69.4 ± 1.39
Full-bloom	2.10	8.6	5.4	10.4	33.0	47.9	3.3	58.0 ± 1.67	58.2 ± 0.50

[a] Standard error of the mean.

advancing maturity of the forage. Methoxyl is believed to be contained largely in the lignin fraction which, in turn, constitutes a portion of the crude fiber and nitrogen-free extract fractions. Since the results of most studies have shown that the crude fiber content of forages increases with advancing stage of growth, the crude fiber values obtained in this study must be regarded with caution Even though these values may be in error because of analytical difficulties, nevertheless, they suggest the inadequacy of crude fiber as an index of the feeding value of forages, as has been reported by many workers (3, 4, 6, 11).

The mean composition of the feces of steers hand-fed and grazing herbage of the same source is summarized in table 2. These data show that the fecal concentrations of crude protein and ether extract decreased, while that of methoxyl increased with advancing age of the plant consumed. Since the level of lignin was higher in the feces of steers fed the most immature forage than in that of steers receiving the more mature forages, it seems possible that a given quantity of lignin may depress the digestibility of other plant constituents to a greater extent in more mature plants than it does in immature ones. If this is true, the nature of the lignin present may influence digestibility to a greater extent than the amount of lignin *per se*. Zherebov (15), Phillips and Goss (9) and others have reported that the methoxyl content of lignin increases with advancing maturity. The addition of methoxyl groups to lignin may change its nature so that it has a greater depressing action on digestibility. It is recognized that other

chemical and structural changes in lignin associated with the age of the plant may be involved in lignin's influence upon the feeding value of plant materials.

TABLE 2

Mean composition of feces (dry basis)

Methoxyl	Lignin	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
(%)	(%)	(%)	(%)	(%)	(%)	(%)
Hand-fed steers						
			Vegetative stage			
2.59 ± 0.02[a]	23.9 ± 0.78	11.3 ± 0.36	15.7 ± 0.21	38.4 ± 0.89	26.3 ± 0.34	8.2 ± 0.21
			Boot-to-early-head stage			
2.91 ± 0.03	20.8 ± 0.27	8.3 ± 0.26	12.1 ± 0.35	30.3 ± 0.10	43.5 ± 0.65	5.7 ± 0.24
			Full-bloom stage			
3.43 ± 0.04	22.3 ± 0.27	6.8 ± 0.24	9.8 ± 0.18	32.7 ± 0.98	45.2 ± 0.36	5.5 ± 0.25
Grazing steers						
			Vegetative stage			
2.29 ± 0.09	21.3 ± 0.74	11.2 ± 0.35	17.3 ± 0.70	30.6 ± 3.42	31.2 ± 3.70	9.6 ± 0.59
			Boot-to-early-head stage			
2.77 ± 0.12	21.3 ± 1.37	9.7 ± 0.44	13.6 ± 0.38	31.7 ± 2.79	38.4 ± 3.70	6.6 ± 0.27
			Full-bloom stage			
3.28 ± 0.11	21.8 ± 0.46	7.9 ± 1.26	10.7 ± 0.78	30.7 ± 1.42	44.4 ± 1.47	6.3 ± 0.84

[a] Standard error of the mean.

The mean apparent digestibility of various constituents of the hand-fed herbage is shown in table 3. The coefficients for methoxyl, crude protein and nitrogen-free extract decreased successively with approaching maturity of the herbage. The negative coefficients shown for lignin merely mean that under the conditions of this experiment the recovery of the consumed lignin from the feces was greater than 100 per cent. If it is assumed that lignin is completely indigestible, either the lignin values obtained for the forages were too low or those determined for the feces were too high. Since good agreement with other laboratories was ob-

TABLE 3

Digestibility of some constituents of hand-fed forage at three stages of plant growth

Methoxyl	Lignin	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
(%)	(%)	(%)	(%)	(%)	(%)
		Vegetative stage			
55.2 ± 0.55[a]	− 14.1 ± 4.23	73.7 ± 0.28	72.8 ± 0.56	78.7 ± 0.35	53.6 ± 1.71
		Boot-to-early-head stage			
43.5 ± 0.78	− 8.2 ± 2.21	66.1 ± 0.91	73.5 ± 0.76	61.6 ± 1.25	56.1 ± 0.47
		Full-bloom stage			
31.4 ± 3.41	− 9.3 ± 3.08	60.8 ± 1.00	58.3 ± 2.67	60.5 ± 1.44	30.3 ± 2.02

[a] Standard error of the mean.

tained with forage check samples from Ellis' laboratory (5), it is considered more likely that the error is in the analysis of the feces. It follows that possibly some interfering substance in the feces was included in the "lignin" residue which either was not included or was included to a smaller extent in the lignin residue isolated from the forages.

In order to examine the relationships of various constituents to each other and to the dry matter digestibility of the forages, correlation coefficients were determined (12) for both the forage and fecal constituents. These are shown in tables 4 and 5, respectively. The data in table 4 indicate that as the level of both

TABLE 4

Correlation coefficients of the amounts of constituents of pasture grass with each other and with the amount of digestible dry matter

	Lignin	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Digestible dry matter
Methoxyl	1.000	−0.901	−0.936	1.000	−1.000	−0.991
Lignin		−0.893	−0.942	0.999	−1.000	−0.989
Crude protein			0.691	−0.910	0.893	0.950
Crude fiber				−0.928	0.943	0.882
Nitrogen-free extract					−0.999	−0.994
Ether extract						0.989

Level of significance with 3 observations, 5% =0.997, 1% = 1.000.

TABLE 5

Correlation coefficients of the amounts of feed constituents with each other and with amount of digestible dry matter in the forage

	Lignin	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Digestible dry matter
Methoxyl	−0.338	−0.953	−0.547	0.826	−0.785	−0.974
Lignin		0.552	−0.714	−0.707	0.788	0.425
Crude protein			0.710	−0.941	0.920	0.962
Crude fiber				−0.899	0.800	0.561
Nitrogen-free extract					−0.959	−0.844
Ether extract						0.850

Level of significance with 9 observations, 5% = 0.666, 1% = 0.798.

lignin and methoxyl increased in the hand-fed forage, the crude protein and ether extract contents and the dry matter digestibility decreased.

In a consideration of fecal constituents (table 5) it was found that the lignin level was inversely related to the crude fiber and nitrogen-free extract contents, but was not correlated significantly with the crude protein content of the feces or with the forage dry matter digestibility. However, a high negative correlation of the methoxyl content of the feces with the level of crude protein in the feces and with the dry matter digestibility of the hand-fed forage was found. The methoxyl and nitrogen-free extract contents of the feces were correlated positively.

Because of the high positive correlation between the crude protein content of

the feces and the digestibility of the dry matter (0.962) and the high negative correlation between the methoxyl content of the feces and dry matter digestibility (−0.974), a multiple regression (12) was calculated of the digestible dry matter on the methoxyl (X_1) and the crude protein (X_2) contents of the feces. The multiple correlation coefficient was 0.980 and the multiple regression equation was $Y = 87.82 - (11.32\ X_1 - 0.926\ X_2)$. Further investigations of this relationship may show that the digestibility of forage material may be predicted with some degree of accuracy by a combination of methoxyl and crude protein determinations on fecal material.

The variable selectivity of grazing animals makes it difficult if not impossible to evaluate pastures directly. That the value of pasture herbage selected by grazing animals is different from that of the whole, clipped plant is suggested by the differences in the digestibility of dry matter shown in table 1. Further differences in the composition of the whole, clipped herbage and that selected by the grazing animals are suggested by the data on fecal composition shown in table 2. Consequently, it is clear that the information obtained on pasture forage in a hand-feeding trial is not applicable to the same forage selectively grazed. It is obvious also that forage samples taken manually are likely to be of a composition different from that selected by grazing animals.

It is possible that some constituent may be excreted in the feces in a manner related quantitatively to the digestibility or to some other criterion of the feeding value of forages. Such a relationship would obviate the difficulties introduced by selective grazing and by manual sampling of forage. An attempt to circumvent these problems using plant pigments absorbing light at 406 mμ has been reported (11). Observations of the data of Forbes and Garrigus (6, 7) and of others suggest that the level of lignin in the feces also might be employed in a similar manner to avoid the errors resulting from selective grazing when the usual lignin-ratio technique is employed, although, apparently because of difficulties in the analysis of feces for lignin, our data did not show a satisfactory relationship.

Since methoxyl constitutes a portion of the lignin complex, an examination was made of the relationship (fig. 1) between the level of methoxyl in the feces and the digestibility of the forage dry matter. The relationship obtained as a result of hand-feeding and grazing trials is expressed by the equation $Y = 117.89 - 17.62X$, where Y = per cent of digestibility of forage dry matter and X = per cent of methoxyl in feces (dry basis). The standard error of estimate of the digestibility of the consumed forage was 1.80 per cent. The digestibility of the forage consumed by grazing steers was determined by the fecal-chromogen method (11), while the digestibility of the whole, clipped forages was determined in conventional digestion trials. It is interesting that this relationship for grazed forage agreed well with that for the whole, clipped forage. Although these data are not sufficient to evaluate the accuracy with which digestibility may be predicted from the fecal methoxyl level, they do suggest that this relationship is promising particularly in the study of forage under conditions of grazing.

Should further study show that the relationships described in this report exist

for other kinds of forage, methoxyl may become at least as reliable an index of the feeding values of forages as lignin. The use of methoxyl has a further advantage in that it is relatively easily measured.

It would be more desirable, however, if a greater change in the methoxyl content of feces per unit change of forage digestibility occurred. Further limitations to the use of methoxyl groups as an index are associated with the method employed to measure these groups. Occasionally the end point of the titrimetric analysis is obscure and the method does not discern between the methyl groups of lignin and those of other substances or between ethoxyl and methoxyl groups. However, the ethoxyl content of forages is relatively small as compared to the amount of methoxyl. Attention is being given to these limitations at the present time.

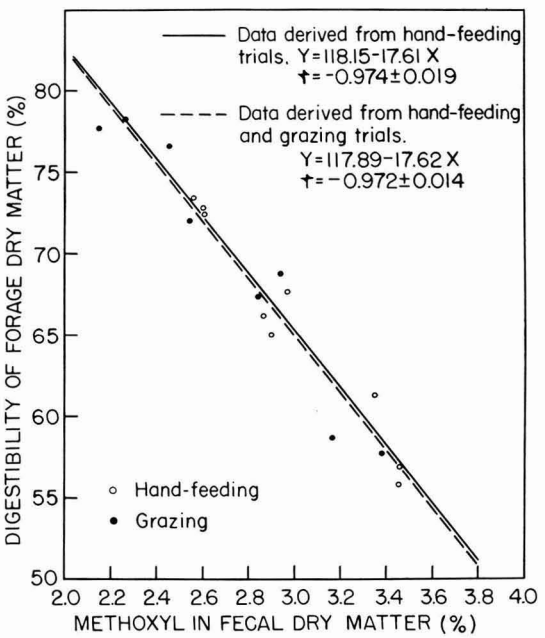

FIG. 1. Relationship of methoxyl level of feces to forage digestibility.

In these studies it was found that a considerable portion of the methoxyl consumed disappeared from the plant material as it passed through the digestive tract of steers. Perhaps most of the apparently digested methoxyl was associated with substances other than lignin, although some of it may have been split off the lignin complex. The recent report of Forbes and Garrigus (7) showing that the proportion of lignin consisting of methoxyl is the same in forage as in feces supports this viewpoint. It generally is believed that most of the methoxyl groups present in plant materials are associated with lignin (9, 10). The high positive correlations obtained between the lignin and methoxyl contents of the forages used in this study tend to support this viewpoint. As the plant becomes

more mature, a greater proportion of lignin consists of methoxyl (9, 10). The decreasing digestibility of methoxyl with approaching maturity of the plant observed in this study may mean that more methoxyl is associated with the indigestible lignin in more mature forages.

SUMMARY

The relationship of the methoxyl content of forage and of feces to the digestibility and to the content of crude protein, crude fiber, ether extract and nitrogen-free extract has been studied at three different growth stages of pasture herbage. The relationship of the lignin to the methoxyl content and to the contents of the proximate constituents of the plant also have been studied.

The digestibility of forage dry matter was correlated negatively with the amount of lignin (−0.989) and of methoxyl (−0.991) in the forage consumed. A highly significant negative correlation (−0.974) also existed between the methoxyl content of the feces and the digestible dry matter content of the forage. The correlation (0.425) between the lignin content of the feces and amount of digestible dry matter in the forage consumed was not significant.

The crude protein content of the feces also was correlated very highly with the digestible dry matter content of the forage (0.962).

This study indicates that the methoxyl content of forages and/or feces may prove, with further study, to be equal or superior to the lignin content as an index of the digestibility of forages. If this should prove to be valid for all forages, the analytical task would be simplified because the procedure for the determination of methoxyl is simpler than that for lignin. In addition, when methoxyl is determined, a distinct chemical radical is measured. This is not true in the determination of lignin by present methods.

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BOVINE PROTEIN-BOUND SERUM IODINE AND ITS RELATION TO AGE AND BREED

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With the recent high level of interest in the feeding of iodinated protein to dairy cattle, the conclusion has been reached that increased response in milk production is obtained, at least temporarily with some cows, while others do not respond. The review by Blaxter *et al.* (3) covers the voluminous literature up to 1949 in a critical manner. Since that date, work substantiating this conclusion has been reported by Reece (8) and Thomas *et al.* (9). Blaxter (2) reported a breed difference to exist in the response to iodinated casein feeding among British cattle when fed a standard amount per individual. Some of this difference was ascribed to variation in body weight. The feasibility of indiscriminately feeding iodinated protein when the response is unpredictable has been questioned (5, 6). Measuring the thyroid activity to increase the predictability of a given response appears not to have been tried.

A number of tests have been devised for the purpose of measuring thyroid activity. One, which is coming into common use in the field of human medicine, is the protein-bound iodine test (PBI). This test measures the level of iodinated protein in the serum, which under normal conditions of diet is made up principally of the thyroid hormone together with its related substances (7).

There are some very definite advantages of the protein-bound serum iodine test over the basal metabolism test in studies of thyroid activity. The basal metabolism test measures the oxygen consumption of the body, which is the end result of all the oxidative processes of the body. This rate of oxygen consumption may or may not be related to the transport, utilization and breakdown of the thyroid hormone. According to Rapport *et al.* (7), the oxygen consumption of the body may be influenced by such diverse non-thyroid hypermetabolic states as essential hypertension, cardiac failure, carcinoma, leukemia and other blood dyscrasias, osteitis deformans, certain infectious diseases and psychic disturbances; or by hypometabolic states such as Addison's disease, Simmonds' disease, or other pituitary dysfunctions and anorexia nervosa. With domestic animals, the increased oxygen consumption due to psychic disturbances while making a basal metabolism determination, or the necessity of using only well trained animals, poses a real difficulty in collecting data on large numbers of animals. Added to this problem is the rarity of finding lactating ruminants in a post absorptive state. This difficulty is eliminated by the use of the protein-bound serum iodine test.

In initiating a study of thyroid activity in cattle, it was decided first to determine the normal range of protein-bound serum iodine in cattle under known environment and ascertain the extent to which certain factors influence its level in the serum. The effect of age and breed will be considered in this paper.

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EXPERIMENTAL PROCEDURE

The animals used in the experiment were from the Ohio State University Dairy and Beef Herd and the Veterinary Clinic. The environment of the dairy cows was similar throughout the herd. They were kept together as a group in the same pasture, lots and barn. The feed for the animals came from the same sources. All animals had access to iodized salt in the block form while on pasture and had it mixed in with their feed while in the barn.

The environment of the beef cattle may have differed from that of the dairy cattle in that they were kept in separate pastures, lots and barns and were under separate management.

The blood samples were drawn during the period from October, 1950, to March, 1951. Ten to 20 ml. of blood were required for an analysis. An average of ten samples was drawn and analyzed each week.

The samples were analyzed in the Iodine Laboratory of the Department of Surgical Research, according to the method developed by Conner *et al.* (4). The method consists of the precipitation of the protein of the serum (including the protein-bound iodine) by addition of solutions of $ZnSO_4$ and NaOH. The material then is centrifuged. The supernatant fluid (containing the soluble inorganic iodine) is decanted off, thus effecting a separation of inorganic iodine from protein-bound iodine. The precipitated protein material (with the PBI) is oxidized with sulfuric and chromic acids, followed by two boiling washings with distilled water. A reducing agent (phophorus acid) then is added to the oxidized material and the released iodine is distilled into a solution of KOH. Measured quantities of arsenious acid, HCl and ceric sulfate then are added to the distillate imparting to it a deep yellow color. The rate at which this solution decolorizes (as measured by an Evelyn photoelectric colorimeter) indicates the quantity of iodine present. Since the rate of the decolorization is directly proportional, within limits, to the quantity of iodine present, the comparison of the amount of decolorization in a given period of time of an unknown quantity of iodine with the decolorization produced by known quantities of iodine indicates the quantity present. Recovery experiments using beef serum plus 10 per cent whole blood show about 100 ± 5 per cent recovery, with an error of over 10 per cent being infrequent (3). The results of the analysis were recorded in terms of micrograms of iodine per 100 ml. of serum.

RESULTS AND DISCUSSION

In regard to the influence of age, it was noticed early in the experiment that calves were higher in PBI than mature cows. When the PBI values were plotted against age, a definite trend was noticed, as shown in figure 1. This decline of PBI with age in cattle compares somewhat favorably with the decline in basal metabolic rate with age which has been noticed in humans (1).

Upon analysis of the data by breed, definite differences were found to be present (table 1). An analysis of variance showed either significant or highly significant differences to exist between certain of the breeds. The Jersey breed, for example, differed significantly from the Guernsey, Brown Swiss and Ayrshire

breeds and highly significantly from Holsteins and the combined beef breeds. Jerseys averaged the highest, followed by Guernsey, Brown Swiss, Ayrshire, Holstein and beef breeds.

The effect of age on the PBI does not explain the breed differences. In fig. 1, the Jersey values, in general, are seen to be above the mean, while the Holstein values were below the mean for almost any age group. A correction factor for

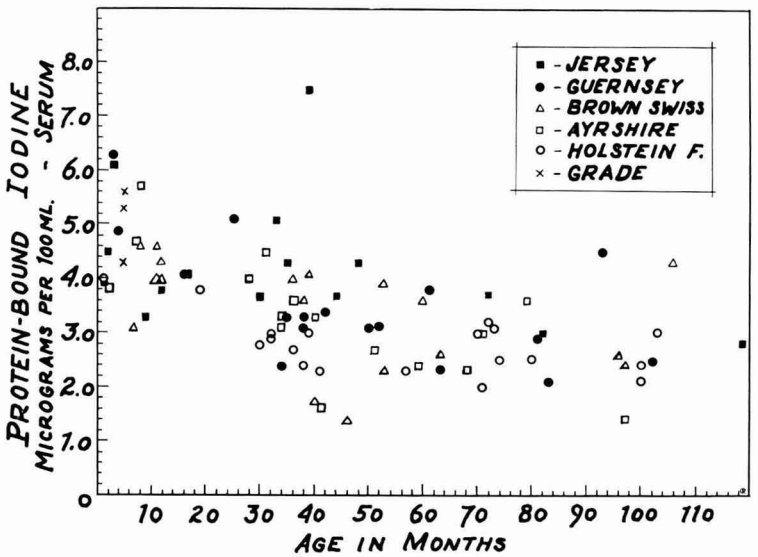

FIG. 1. Change in protein-bound iodine values with age.

TABLE 1

The significance of breed as related to the protein-bound serum iodine in the bovine female

Breed	No.	PBI	Std. dev.	Range	Breeds with significantly smaller means[a]	
					5% level	1% level
		(γ %)				
Jersey	17	4.11[b]	1.21	2.6–7.5	G., B.S., A.	H., Beef
Guernsey	18	3.51	1.19	2.1–6.3	H.	Beef
Brown Swiss	21	3.37	0.96	1.4–5.0	H.	Beef
Ayrshire	20	3.19	1.05	1.4–5.7		Beef
Holstein-F.	20	2.73	0.55	1.8–4.0		
Beef breeds[c]	20	2.19	0.58	1.3–4.7		

[a] Grateful acknowledgment is made to D. R. Whitney, Statistical lab., Ohio State Univ., for test of significance.

[b] Values are for individual cows; where more than 1 sample/cow was analyzed, the values were averaged.

[c] Hereford-7, Angus-7 and Shorthorn-6.

age was calculated from the mean shown on the graph. This then was applied to the PBI values for the cattle (table 2). Correcting for age produced no change in rank of the breeds in this particular case, since the animals of different breeds

TABLE 2

Breed	PBI's not corrected for age	PBI's corrected for age[a]
	(γ %)	(γ %)
Jersey	4.11	4.19
Guernsey	3.51	3.42
Brown Swiss	3.37	3.24
Ayrshire	3.19	2.95
Holstein	2.73	2.90

[a] Beef values could not be corrected accurately since their ages in many cases were known only in terms of years. However, since their average age was about 3 yr. and the cows were corrected for age to a 3- to 3.5-yr. basis, they were considered to be roughly comparable.

were of the same relative age groups. The range in uncorrected individual values for the entire group tested was from 1.3 to 8.6 γ per cent.

SUMMARY AND CONCLUSIONS

Protein-bound serum iodine (PBI) determinations were performed on 116 dairy and beef cattle of the Ohio State University Herd and Veterinary Clinic.

It was found that the age of the animal has a pronounced influence on PBI. The younger the animal, in general, the higher the PBI.

The breed differences found show that the PBI value is affected by the genotype of the cattle. Significant and highly significant differences were found to exist between certain of the breeds. There were too few grade cattle included to allow for an interpretation of their probable genotypes. With the cattle studied, the breeds ranked from high to low as follows with regard to their average PBI: Jersey, Guernsey, Brown Swiss, Ayrshire, Holstein and beef breeds.

The large difference between the beef and dairy breeds is not attributed entirely to inheritance, since the possibility of all sizable environmental differences has not been ruled out.

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BLOOD LEVELS OF ASCORBIC ACID AND VITAMIN A DURING VITAMIN A DEPLETION AND EFFECT OF ADMINISTRATION OF ASCORBIC ACID DURING TERMINAL VITAMIN A DEPLETION IN THE DAIRY COW[1]

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Decreases in blood plasma levels as well as lowered urinary excretion of ascorbic acid have been reported to accompany vitamin A depletion (1, 11, 12, 13). Although a relationship between the blood levels of ascorbic acid and spinal fluid pressure in the calf was postulated (1), later work (11, 13) showed such a relationship to be non-existent.

Work with laboratory animals, primarily the rat, on unrestricted feeding has indicated a decrease in ascorbic acid concentration of various tissues in severe vitamin A depletion (9, 10). However, using restricted feeding, Mapson and Walker (9) were unable to demonstrate a specific relationship between the ability to synthesize ascorbic acid and the vitamin A status of the rat.

Boyer *et al.* (1) reported a correlation between plasma levels of ascorbic acid and vitamin A, but since feed refusals by the dairy calf, when fed a controlled intake of a vitamin A depletion ration, have been found to occur only when the blood plasma level of vitamin A had decreased to less than 4.0γ per cent (3), this correlation may not have been due to vitamin A *per se* but to plane of nutrition and/or anorexia. Further, with the establishment of the sensitivity of the bovine parotid gland to hypovitaminosis A, characterized by the development of squamous metaplasia (7), the need to use this histopathological change as a diagnostic tool to re-examine the possible role of ascorbic acid in vitamin A depletion was apparent.

The purpose of this study was to determine the effects of controlled feeding of a vitamin A-free ration on blood levels of ascorbic acid and the relationship of ascorbic acid to vitamin A as demonstrated by the possible role of ascorbic acid in alleviating characteristic histological and clinical changes occurring during terminal vitamin A depletion.

EXPERIMENTAL

Animals. Eighteen male calves, nine Guernseys and nine Holsteins, 105 days of age, were placed on experiment during the month of January through May,

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1951. They were stabled in individual tie-stalls where the temperature was maintained at a minimum of 10° C. All calves had been raised to 105 days of age on a limited-whole milk dry calf starter regime in which the only variable was the form of alfalfa hay fed (4). Each calf was fed a depletion ration, which was identical in content to that reported previously (3), according to the following formula: $Y = 0.0561\ W^{0.87}$ (where Y = the pounds of feed required daily and W = the weight of the calf in pounds). It was derived using Morrison's mid-interval T.D.N. requirements for 300-lb. growing dairy cattle (15) and Brody's calculation (2) of Morrison's suggested weight relationship for the maintenance requirement of dairy cattle. The amounts of the depletion ration fed were adjusted to the weight of the individual calves at successive 7-day intervals.

When the blood plasma vitamin A level had decreased to less than 4.0γ per cent for 2 consecutive wk., calves were considered depleted of their vitamin A stores (6). They then were subjected to one of three treatments: (a) Slaughter (control group); (b) subcutaneous injections of ascorbic acid[2] at the rate of 2 g. daily for 14 days, followed by slaughter; and (c) oral administration of vitamin A in the form of fish liver oil[3] at a daily rate of 200,000 U.S.P. units for 14 days, followed by slaughter. The assignment of calves to a particular treatment was done randomly with restriction as to breed.

Observations and analyses. Daily feed intakes and refusals were weighed to the nearest 0.1 lb. On the 105th day and at 7-day intervals thereafter, liveweight was recorded and venous blood samples were drawn. After the blood samples were obtained, they were immediately chilled. An aliquot for whole blood ascorbic acid determination was removed and the remaining blood centrifuged. A measured aliquot of plasma for vitamin A determination was held at –18° C. for carotene and vitamin A analyses and a measured amount of the blood and plasma for ascorbic acid immediately deproteinized. The acid filtrate then was stored in the dark at 2° C. for subsequent analysis.

Daily clinical observations were recorded. Spinal fluid pressures were determined at the start of the experiment, at 4-wk. intervals thereafter until the calf was depleted of its vitamin A stores and just prior to slaughter. At slaughter, tissues were taken from the adrenal, colon, eye, heart, kidney, liver, lung, parotid, pituitary, small intestine, testes and thyroid for histopathological examination and the liver for carotene and vitamin A analyses. Analytical, histological and statistical procedures were similar to those previously reported (3). Both whole-blood and plasma ascorbic acid were determined by the method of Roe and Keuther (16).

RESULTS AND DISCUSSION

The results have been analyzed from three points of view: (a) During initial vitamin A depletion, that is until the blood plasma vitamin A level for each calf

[2] Crystalline U.S.P. ascorbic acid (Merck) dissolved in 30 ml. H_2O containing 3.5 g. $Na_3PO_4 \cdot 12\ H_2O$.

[3] The fish liver oil contained 25% by weight of crude soybean lecithin and 27,000 U.S.P. units of vitamin A per g. It was generously supplied by the Nopco Chemical Co., Harrison, N. J.

decreased to less than 4.0γ per cent (fig. 1); (b) during terminal vitamin A depletion, that is for the 2 wk. immediately following the above (table 2) so as to establish quantitative data for subsequent assessment of the possible effects of the

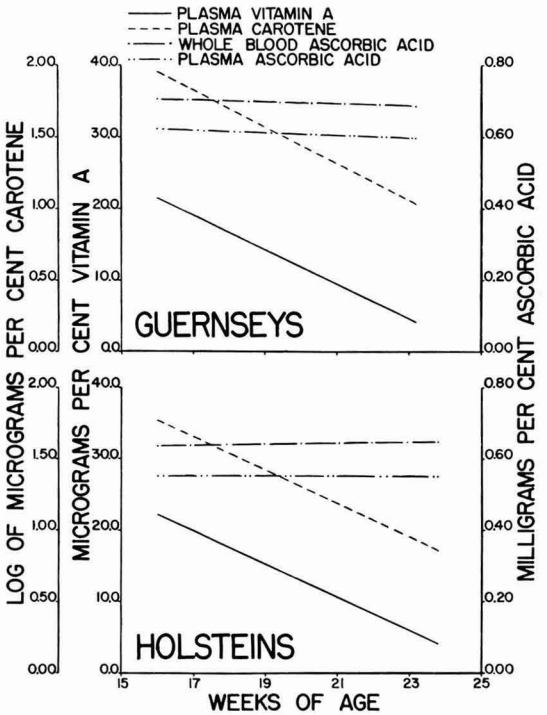

FIG. 1. The effect of vitamin A depletion to blood plasma vitamin A levels of 4γ% on regression of whole blood ascorbic acid and plasma ascorbic acid, carotene and vitamin A on age of Guernsey and Holstein calves.

administered ascorbic acid; and (c) treatment of terminal vitamin A depletion with subcutaneously administered ascorbic acid (tables 1 and 2).

Feed consumed. During a 2-day period, two calves with diarrhea accompanied by rectal temperatures above 103° F. did not entirely consume the ration allowed. Another calf which previously had demonstrated a dislike for grain did not consume the entire ration allowance until 120 days of age. All other calves readily consumed the depletion ration fed during the initial vitamin A-depletion period.

During the terminal vitamin A-depletion period (table 1) three calves did not consume all feed allowed. Feed intake decreased progressively during the period of subcutaneous injections of ascorbic acid, whereas the oral administration of vitamin A resulted in complete consumption of feed allowed. These results demonstrated the inability of subcutaneous injections of ascorbic acid to alleviate the decrease in feed intake characteristic of terminal vitamin A depletion in the

TABLE 1

Effect of vitamin A depletion on terminal values of feed consumed, liveweight, plasma vitamin A, whole blood and plasma ascorbic acid and spinal fluid pressure and of subsequent administration of ascorbic acid and vitamin A on these criteria

Exptl. group	Weeks before slaughter							L.S.D.[a] between weeks
	5	4	3	2	1	0		
				Per cent of feed consumed				
Controls	100.0	100.0	99.8	100.0	.....	.....		0.3
Ascorbic acid	100.0	100.0	100.0	99.2	96.5	92.9	(93.5)*[b]	4.7
Vitamin A	99.4	100.0	100.0	99.9	99.9	100.0	(100.0)	1.2
L.S.D. between groups[a]	1.9	0.0	0.5	2.9	10.0	3.7		
				Log of liveweight (*lb.*)				
Controls	2.44	2.47	2.48	2.51	.....	.....		0.04
Ascorbic acid	2.48	2.50	2.52	2.52	2.53	2.54	(2.56)	0.11
Vitamin A	2.49	2.51	2.54	2.54	2.56	2.58	(2.57)	0.09
L.S.D. between groups	0.09	0.09	0.08	0.09	0.11	0.10		
				Plasma vitamin A (γ%)				
Controls	6.7	5.0	3.0	1.9	.....	.....		0.9
Ascorbic acid	6.7	4.8	3.2	2.1	1.6	1.6	(1.4)**	0.7
Vitamin A	6.6	5.3	3.4	2.3	41.5	39.7	(40.0)	2.7
L.S.D. between groups	1.6	0.9	0.8	0.5	4.3	2.1		
				Whole blood ascorbic acid (*mg.* %)				
Controls	0.72	0.73	0.70	0.65	.....	.....		0.08
Ascorbic acid	0.59	0.62	0.58	0.52	0.95	0.88	(0.88)*	0.18
Vitamin A	0.68	0.67	0.64	0.56	0.69	0.63	(0.62)	0.10
L.S.D. between groups	0.09	0.10	0.08	0.13	0.22	0.23		
				Plasma ascorbic acid (*mg.* %)				
Controls	0.63	0.65	0.60	0.52	.....	.....		0.11
Ascorbic acid	0.48	0.52	0.46	0.40	0.90	0.73	(0.75)*	0.17
Vitamin A	0.57	0.58	0.54	0.45	0.54	0.50	(0.48)	0.12
L.S.D. between groups	0.11	0.12	0.13	0.14	0.23	0.21		
				Spinal fluid pressure (*mm.* H_2O)				
Controls				247		.....		.....
Ascorbic acid				211		269	(271)*	86
Vitamin A				220		177	(175)	68
L.S.D. between groups				90		89		

[a] Least significant difference between means at $P < 0.05$.

[b] Values in parentheses are adjusted 0 day or wk. treatment means which were adjusted by covariance for the initial value at 2-wk. prior to slaughter before administration of either ascorbic acid or vitamin A. * indicates significance at $P < 0.05$ and ** at $P < 0.01$.

young dairy calf and confirmed previous work (3) on the effect of vitamin A depletion on feed consumption.

Liveweight. All calves made rapid gains in liveweight until the blood plasma level of vitamin A decreased to 4γ per cent. During this period regression equations of the square root of liveweight (y) on weeks of age (x) for Guernseys was $Y = 7.0 + 0.4194x$ and for Holsteins $Y = 8.3 + 0.4768x$. The rate of increase in liveweight for Holstein calves was greater than for Guernsey calves.

After the blood plasma level of vitamin A decreased to 4γ per cent, there was a tendency for the growth rate to diminish (table 1). Oral administration of vitamin A resulted in a greater increase in liveweight than did subcutaneous injection of ascorbic acid. The difference was not statistically significant, however.

Blood levels of ascorbic acid and vitamin A. During the initial vitamin A-depletion period, ascorbic acid levels in whole blood and plasma (fig. 1) exhibited no significant change, while both plasma carotene and vitamin A decreased. The rates of change (linear regression coefficients) of whole blood and plasma ascorbic acid were -0.0001 ± 0.0021 and -0.0015 ± 0.028 mg. ascorbic acid per 7-day period, respectively. Rates of change of the log of plasma carotene concentration were -0.1326 ± 0.0042 for Guernsey calves and -0.1174 ± 0.0112 for Holsteins. Similar values for plasma vitamin A were $-2.3612 \pm 0.1132\gamma$ per 7-day period for Guernseys and -2.2978 ± 0.1462 for Holsteins.

TABLE 2

Effect of vitamin A depletion and of subsequent subcutaneous ascorbic acid and oral vitamin A administrations on liver storage of carotene and vitamin A and on parotid gland histology

Exptl. no.	Breed	Liver			Squamous metaplasia in the parotid gland inter-lobular ducts and main duct
		Weight	Carotene	Vitamin A	
		(*g.*)	(γ/*g.*)		
			Controls[a]		
F. P. 21	G	2,364	0.16	0.05	+
F. P. 24	G	2,379	0.64	0.06	−
F. P. 18	G	2,360	1.10	0.04	+
F. P. 33	H	2,222	0.18	0.18	+
F. P. 31	H	2,840	0.16	0.10	+
F. P. 35	H	2,700	0.24	0.02	+
Mean		2,478	0.41	0.08	
			Subcutaneous ascorbic acid[b]		
F. P. 17	G	2,358	0.36	0.01	+
F. P. 16	G	2,452	0.30	0.02	+
F. P. 19	G	2,540	0.36	0.00	+
F. P. 34	H	2,700	0.20	0.06	+
F. P. 36	H	3,238	0.38	0.06	+
F. P. 28	H	3,460	0.20	0.03	+
Mean		2,791	0.30	0.03	
			Oral vitamin A[c]		
F. P. 23	G	1,945	0.04	56.04	−
F. P. 20	G	2,519	1.37	34.71	T
F. P. 22	G	2,259	1.05	46.36	T
F. P. 30	H	2,714	1.90	25.12	−
F. P. 29	H	3,483	0.18	25.60	+
F. P. 32	H	3,506	0.60	21.50	T
Mean		2,738	0.86	34.89	

[a] Slaughtered after blood plasma vitamin A decreased to less than 4γ% for 2 consecutive wk.
[b] Slaughtered after blood plasma vitamin A decreased to less than 4γ% for 2 consecutive wk. followed by daily subcutaneous injections of 2 g. of ascorbic acid for 14 d.
[c] Slaughtered after blood plasma vitamin A decreased to less than 4γ% for 2 consecutive wk. followed by daily oral administration of 200,000 U.S.P. units of vitamin A for 14 d.

Upon reaching 4γ per cent, the plasma vitamin A level decreased at a slower rate than previously (table 1). Whole blood and plasma levels of ascorbic acid tended to decrease. The latter, prior to either ascorbic acid or vitamin A administration, (see second week before slaughter, table 1) were in some instances sig-

nificantly lower than those observed earlier. Oral administration of vitamin A caused a marked elevation of this substance in the plasma which was accompanied by slight but not significant increases in concentration of ascorbic acid in the whole blood and plasma. Subcutaneous injections of ascorbic acid caused a noticeable increase in the ascorbic acid content of whole blood and plasma but were without effect on plasma vitamin A.

It was apparent that in early A-hypovitaminosis uncomplicated by differences in feed intake, ascorbic acid concentration in whole blood and plasma was not affected. In terminal or severe vitamin A depletion, ascorbic acid concentration of the blood did decrease and the data were in agreement with the literature (1, 13). The influencing factor in the decrease in ascorbic acid concentration may be diminished feed intake rather than lack of vitamin A *per se*. This factor needs to be investigated in the calf by the excellent method that Mapson and Walker (9) used in the rat.

Spinal fluid pressure and clinical observations. Since in this study spinal punctures were done at 4-wk. intervals, the data for spinal fluid pressure corresponding to plasma vitamin A concentrations between 10 and 4γ per cent were incomplete. From the literature (11, 14) and from unpublished data (5), it is evident that spinal fluid pressures increase in rapidly growing male calves at blood plasma levels of vitamin A considerably greater than 4γ per cent.

At blood plasma levels of vitamin A of less than 4γ per cent, spinal fluid pressures were above 200 mm. H_2O (table 1). Oral administration of vitamin A caused a decrease in spinal fluid pressure. In contrast, subcutaneous administration of ascorbic acid had no effect and the levels increased as would be expected in prolonged terminal vitamin A depletion. These data confirmed Moore's work (13) on the lack of association between the ascorbic acid status and increased spinal fluid pressure of the calf.

Clinical symptoms, with the exception of diarrhea accompanied by rectal temperatures above 103° F. in two calves, were not apparent until the blood plasma level of vitamin A decreased to 4γ per cent. After this level was obtained, muscular incoordination in ten calves, diarrhea not accompanied by elevation in rectal temperature in six, exopthalmus in one and convulsions in one were observed. In those calves receiving subcutaneous injections of ascorbic acid, the degree of muscular incoordination and diarrhea became progressively greater. As expected, oral administration of vitamin A resulted in disappearance or alleviation of these symptoms.

Slaughter data. Squamous metaplasia characteristic of terminal vitamin A depletion (7) was observed in the interlobular ducts and the main duct of the parotid gland (table 2) of five or six control calves and of all calves receiving subcutaneous injections of ascorbic acid. By contrast, of those calves receiving 200,000 U.S.P. units of vitamin A daily for 14 days, only one showed squamous metaplasia, two showed no lesions and three showed a transitional type epithelium. This finding substantiated earlier data on the specificity of the parotid gland lesion for A-hypovitaminosis (3, 7) and demonstrated the reversibility of the lesion by vitamin A *per se* and the complete ineffectiveness of ascorbic acid

to reverse the lesion. Other tissues examined failed to reveal consistent histological changes and therefore were considered not to be related to terminal vitamin A depletion.

Liver stores of vitamin A were markedly increased by oral administration of this substance (table 2). As would be expected with equal dosages per calf without regard to body weight, Guernsey calves stored greater amounts of vitamin A after the 14-day administration than did Holstein calves.

SUMMARY

Eighteen calves, nine Guernseys and nine Holsteins, 105 days of age and previously raised on a limited-whole milk and dry-calf starter system, were fed a vitamin A-depletion ration in amounts adjusted at 7-day intervals for liveweight.

During initial vitamin A depletion, from 105 days of age until each calf's blood plasma level of vitamin A decreased to less than 4.0γ per cent, there was no significant decrease either in whole blood or plasma ascorbic acid levels. In contrast, both plasma carotene and vitamin A decreased in a relatively uniform manner.

During terminal vitamin A depletion, after blood plasma levels of vitamin A of less than 4.0γ were reached, there was a decrease in consumption of the feed allowed, accompanied by decreases in blood levels of ascorbic acid as well as initial appearance of clinical symptoms of vitamin A deficiency. In addition, spinal fluid pressures of greater than 200 mm. H_2O were observed.

Subsequent administration of ascorbic acid subcutaneously for a 14-day period did not alleviate the anorexia, the clinical symptoms of terminal vitamin A depletion or the increased spinal fluid pressure. In contrast, administration of vitamin A orally resulted in complete consumption of the feed allowed, significant decreases in spinal fluid pressure and disappearance or alleviation of clinical symptoms. Upon slaughter, all calves given ascorbic acid had squamous metaplasia in the parotid gland ducts, whereas of those calves given Vitamin A, one showed squamous metaplasia, two had no lesions and three had transitional type epithelium.

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THE AMINO ACID COMPOSITION OF THE FAT-GLOBULE MEMBRANE PROTEIN OF MILK[1]

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Research dealing with the protein or proteins found on the milk-fat globule has been conducted by numerous workers over the past half century and yet our knowledge concerning this subject is rather limited. Early workers were of the opinion that the membrane protein was a moiety of the major milk proteins, casein, lactalbumin and lactoglobulin. Hattori (3) offered evidence that the membrane protein differed from these proteins in that his "Haptein" contained only 12 per cent nitrogen. Palmer and his associates (8, 9, 10, 13, 14), in an extensive study of the fat-globule membrane, concluded that the membrane protein differed from all other known milk proteins.

The protein isolated by Palmer and his coworkers agreed rather well in composition with that of Hattori on a nitrogen distribution basis. Both were characterized by a low nitrogen content (about 12 per cent) and a high percentage of humin nitrogen, as compared with casein, lactalbumin and lactoglobulin. Each protein, however, differed in the nitrogen distribution within the basic fraction. The only amino acids determined were arginine, histidine, lysine and cystine. The present report deals with the amino acid composition of the milk fat-globule membrane protein as determined by microbiological assays.

METHODS

Isolation of the membrane protein. The procedure of Olson (7) was used with slight modifications to overcome experimental difficulties which arose. Ten gal. of fresh raw cream containing 40 per cent butterfat were used. The cream was poured into a 50-gal. vat and the vat filled with water at 100° F. The mixture was stirred gently for a few minutes, put through a cream separator and the cream collected. This operation was repeated at least four times. A biuret test was conducted on a sample of the washings after each separation until the final sample showed only a very faint pink color.

The washed cream, with a volume of approximately 10 gal., was placed in a cooler for 2 days, churned in a commercial churn at 52° F. and the buttermilk and washings collected. These were mixed and the pH adjusted to 3.95 with glacial acetic acid and placed in the cooler overnight. Some of the supernatant liquid was siphoned off and the remaining suspension was poured into 4-l. beakers and an amount of acetone equal to the volume in the beaker was added. The acetone precipitated the protein and further supernatant was removed by siphoning and centrifuging at 2,000 rpm. on the International no. 2-type centrifuge.

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The precipitate could not be separated completely by this means and therefore was put through a Sharples supercentrifuge at 40,000 rpm. The precipitate was collected, again mixed with an equal volume of acetone, blended in a Waring Blendor and separated in the International centrifuge.

The dry precipitate was washed with absolute alcohol, washed five times with a 1:4 alcohol-ether mixture and finally with pure anhydrous ether. In each case the mixture was separated at centrifugal speeds at 2,000 rpm. The precipitate then was dispersed in a minimum of distilled water and lyophilized in a Stokes lyophilizing apparatus. The yield from 10 gal. of cream was approximately 4 g.

The above procedure was carried out on two different samples of cream. Sample I had been prepared and stored in the refrigerator for a considerable period of time, while sample II was a fresh preparation.

Phosphorus determination. Fifty-mg. samples were burned in a 22-ml. Parr peroxide bomb. The melt was taken up in distilled water, acidified with HNO_3 and the P precipitated with ammonium molybdate. The P then was determined by titration (1).

Sulfur determination. The protein samples (0.3 g.) were burned in a 22-ml. Parr peroxide bomb and analyzed for S as follows: The residue obtained from combustion was taken up in distilled water, slightly acidified with HCl and a saturated solution of Br added. After expelling the excess Br, the solution was made just acid to methyl orange and the sulfate precipitated with $BaCl_2$. The precipitate was dried and weighed and the S calculated.

Amino acid analysis. Microbiological assay procedures were used for the determination of the amino acid composition of the fat-globule membrane protein. *Lactobacillus arabinosus 17-5* (ATCC 8014) was employed for the determination of methionine, isoleucine and cystine; *Leuconostoc mesenteroides P-60* (ATCC 8042) for phenylalanine, tyrosine, serine, proline, aspartic acid and glycine; and *Streptococcus faecalis* (ATCC 9790) for the estimation of arginine, histidine, leucine, lysine, threonine, tryptophane and valine.

The basal medium described by Stokes *et al.* (12) was employed for the estimation of arginine, histidine, leucine, lysine, threonine, tryptophane and valine; the medium of Barton-Wright (2) for isoleucine, tyrosine, serine, proline, cystine, aspartic acid and glycine; the medium of Henderson and Snell (4) for phenylalanine; and the medium of Horn *et al.* (5) was used for the estimation of methionine. Standard microbiological assay procedure was used throughout. The final volume in the assay tubes was 10 ml. Assays involving the use of *S. faecalis* were incubated 48 hr.; those utilizing *L. arabinosus* and *L. mesenteroides* were incubated for 72 hr. Growth was measured titrimetrically. Assays were conducted in duplicate and in most cases in triplicate and values reported are averages of these determinations.

RESULTS AND DISCUSSION

The results of the preliminary analysis of the two preparations are shown in table 1. These figures are the averages of closely agreeing duplicate values.

It is evident that with the exception of S and P, the two preparations were reasonably similar in composition. Reasons for the wide variation in S and P are not apparent at the present time. The value for N (12.34 to 12.38 per cent) suggests

TABLE 1

Composition of fat-globule membrane protein

Sample	Moisture	Fat	Ash	Nitrogen	S	P
	(%)	(%)	(%)	(%)	(%)	(%)
I	7.61	4.33	2.17	12.34	1.34	0.62
II	8.50	5.22	3.22	12.38	2.02	0.30

that the materials are similar in nature to the protein substances isolated by Hattori (3) and Palmer (8), both of whom obtained N values of approximately 12 per cent.

Table 2 shows the average amino acid composition of the two samples of the

TABLE 2

The amino acid composition of the fat-globule membrane protein and other milk proteins[a]

	Membrane protein		Casein	Lactalbumin	β-Lactoglobulin
	Sample I	Sample II			
	(%)	(%)	(%)	(%)	(%)
Arginine	5.0	7.2	3.8	3.4	2.9
Histidine	1.7	2.1	2.7	1.5	1.6
Lysine	6.1	7.5	8.2	10.8	11.4
Tyrosine	3.2	3.2	6.1	2.0	3.8
Tryptophan	0.9	0.9	1.2	1.7	1.9
Phenylalanine	5.2	6.2	4.9	3.6	3.5
Cystine	1.5	1.1	0.4	4.3	2.3
Methionine	2.0	2.1	2.6	1.6	3.2
Threonine	6.4	7.4	4.8	5.4	5.8
Leucine	9.0	8.9	9.2	12.9	15.6
Isoleucine	3.5	4.4	5.6	6.1	8.4
Valine	5.4	6.2	6.9	5.8	5.8
Serine	3.2	4.8	5.9	1.8	5.0
Proline	4.6	4.8	11.6	3.8	4.1
Aspartic acid	4.6	4.9	7.2	9.3	11.4
Glycine	3.0	3.1	2.7	0.4	1.4
Total	65.3	74.8	83.8	74.4	88.1

[a] Anhydrous, ash-free basis.

fat-globule protein isolated in this laboratory. These values are compared with average amino acid values obtained from the literature for the major milk proteins, casein, lactalbumin, and β-lactoglobulin.

Although the two samples of membrane protein were prepared at different times in the laboratory and from different milk sources, the amino acid values were in rather close agreement in most cases. The basic amino acids arginine and lysine and serine showed the widest difference; other amino acids such as phenylalanine and threonine varied as much as 1 per cent, while the remainder showed much closer agreement. Analyses by Wiese and Palmer (14) of the basic amino acids and cystine, although determined chemically, were similar generally

to the values reported in this study. Both cystine and histidine were in the neighborhood of 1 per cent, while arginine and lysine were in the range of 5 to 6 per cent, values approximating those obtained in the present study.

Upon inspection of the amino acid composition of casein, lactalbumin and β-lactoglobulin and comparing these values with those of the fat-globule membrane protein, it becomes evident that little similarity exists between these proteins on an amino acid basis. The membrane protein appears to contain more arginine, phenylalanine, threonine and glycine and less lysine, tryptophan, leucine, isoleucine and aspartic acid than any of the major milk proteins. The remaining amino acids show values intermediate between those of casein, lactalbumin and lactoglobulin. If amino acid composition of a protein may be used as a criterion of protein similarity or dissimilarity, it is obvious that the membrane protein is a distinct type of protein and appears not to be related to the casein, lactalbumin or lactoglobulin on a structural basis.

Past work has shown that various materials are adsorbed on the fat-globule surface, including agglutinin (11) and the enzyme phosphatase (6). Therefore, it is likely that the protein isolated by the present method is not a single molecular substance but a group of proteins, perhaps very similar in chemical properties. The variation in amino acid composition as shown in the present report and by previous investigators (3, 10, 14) possibly is due to the removal of different amounts of the adsorbed protein substances during the washing process. Thus, it is difficult to isolate the "fat-globule membrane protein" in its pure form. An electrophoretic study of the protein material isolated from washed cream may possibly throw some light on this subject.

Because of the small amount present in milk, the membrane protein may be insignificant in its contribution to the nutritive value of the milk proteins. However, on an individual protein basis, it appears to be adequate in the essential amino acids. A short-term growth trial with Wistar albino rats has indicated that the nutritive value of the membrane protein isolated in this study is only slightly less than that of casein.

SUMMARY

Sixteen amino acids have been determined microbiologically on two different samples of the fat-globule membrane protein substance isolated from washed cream. Close agreement in amino acid composition was observed between the two preparations. Lysine and arginine showed the widest variation.

The amino acid composition of the membrane proteins was compared with that of casein, lactalbumin and lactoglobulin. Little similarity was noted between the membrane protein and the major milk proteins which suggests that the former was a unique protein(s) and did not find its origin in either casein, lactalbumin or lactoglobulin.

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THE EFFECT OF AGE AND SIZE ON THE FERTILITY OF DAIRY HEIFERS[1]

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Several reports have indicated that heifers have lower fertility than cows. Whether this is due to a higher percentage of infertile animals or an over-all lower fertility in heifers is not clear. The possibility that age and size influence the fertility of heifers has not been adequately studied. In the following references as well as in the present study, fertility was measured by the percentage of animals conceiving after one, two or three services or by the average number of services required per pregnancy. The records of sterile animals were not omitted from the data.

In a study of 221 heifers and 418 cows, Trimberger and Davis (9) found that 57.5 per cent of the heifers and 61.7 per cent of the cows conceived at first service when bred artificially. Morgan and Davis (5) found that among 537 heifers and 2,090 cows bred naturally, the heifers required 2.52 services per conception and the cows required 2.21 services. Miller and Graves (4) reported that at the end of three services, 67 per cent of the heifers and 73 per cent of the cows had conceived. Olds (7) found that the fertility of heifers was about 2.3 per cent lower than for cows. Davis (1) reported that 53.5 per cent of the heifers had conceived after two services, whereas the average for cows of all ages was 63.8 per cent.

Very little has been published concerning the fertility of heifers bred at various ages and sizes. Tanabe and Salisbury (8) found a rather uniform increase in fertility of cattle from 1 to 4 yr. of age. On the other hand, Gowen and Dove (2) found that fertility decreased rather uniformly from 70 per cent in heifers under 1 yr. of age to 45 per cent for 16-yr.-old cows. White *et al.* (10), after summarizing data for 120 heifers of four breeds, reported that heifers under 15, 15 to 17, 18 to 20, 21 to 23 and 24 mo. of age or over required 2.40, 1.91, 1.60, 2.54 and 2.89 services, respectively, per conception. Olds *et al.* (6), in a study of 435 heifers of the Holstein and Jersey breeds, found a satisfactory fertility in Jerseys 12 to 14 mo. of age; aside from this, the fertiilty increased with age up to 21 to 24 mo. of age. In a study of 1,265 heifers bred artificially, Olds (7) found no significant trends associated with age in the Holsteins and Guernseys, but the fertility of Jersey heifers declined from 64.2 per cent in the group 12 to 15 mo. old to 46.1 per cent in those bred at 24 mo. of age or older. In view of these conflicting results and since it is difficult to secure accurate ages in this type of a field study, it was decided to gather additional records and also to include a chest girth measurement as an estimation of size (3).

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

PROCEDURE

Inseminators in 21 locals of the Kentucky Artificial Breeding Association were asked to record the ages of heifers at the time of first service whenever this information was available. In addition, they were asked to take the chest girth measurement as an indication of size of all heifers bred and to record the data in their herd record books. This was done for a period of 1 yr. (1949–50) and all heifers were allowed at least 4 mo. in which to return for another service before tabulations were made.

RESULTS

Data were obtained for 2,166 heifers of the Holstein, Jersey and Guernsey breeds; ages were obtained for 1,786 heifers and the chest girth measurements for 1,405 heifers. The breeding efficiency of heifers for the various ages is shown in table 1. The Chi-square test indicated that there were no significant fertility differences in the age groups.

TABLE 1

Breeding efficiency of heifers according to age

Age	Holstein		Jersey		Guernsey	
	No. bred	% Non-returns	No. bred	% Non-returns	No. bred	% Non-returns
(*mo.*)						
12–14	36	82.1	154	70.1	67	62.7
15–17	164	68.3	448	70.1	262	63.7
18–20	195	73.9	130	63.1	119	63.9
21–23	51	80.4	28	57.1	21	61.9
24 or over	56	67.9	30	60.0	25	44.0
Unknown	155	69.0	116	63.8	109	62.4
Totals	657	71.7	906	67.6	603	62.5

The breeding efficiency of heifers for the various groups sorted on estimated weights, based on chest measurements, is shown in table 2. Apparently, the dif-

TABLE 2

Breeding efficiency of heifers according to estimated weight as determined from chest girth

Estimated weight	Holstein		Jersey		Guernsey	
	No. bred	% Non-returns	No. bred	% Non-returns	No. bred	% Non-returns
(*lb.*[a])						
301–400	.....	.....	22	63.6	5	60.0
401–500	14	78.6	180	68.3	47	72.3
501–600	77	74.0	230	66.1	134	71.6
601–700	160	68.8	81	70.4	104	61.5
701–800	151	73.5	20	50.0	61	55.7
801–900	69	78.3	.....	.....	12	50.0
901–1,000	34	64.7	2	0.0	2	100.0
Unknown	152	69.7	371	69.0	238	58.0
Totals	657	71.7	906	67.6	603	62.5

[a] Kendrick and Parker (3).

ferences in weights were not closely associated with changes in breeding efficiency, for the Chi-square test indicated that there were no statistically significant differences among the groups.

It should be admitted that complete reliance cannot be placed in all the ages of heifers as given to inseminators by farmers. Some miscalculations or inaccurate estimates are almost certain to occur, although perhaps overestimates of age may tend to cancel the underestimates. Likewise, the chest girth measurements may be subject to error. In one case, a tape measure which had gotten wet was found to have shrunk 3 in. in the span of 72 in. However, in this case, the inseminator recognized the shrinkage promptly and stopped using the tape. Nevertheless, it is believed that, for the most part, the data are reliable and that, within the limits of this study, age and size did not have a marked effect on the fertility of heifers.

SUMMARY

Breeding efficiency was obtained for 2,166 heifers of the Holstein, Jersey and Guernsey breeds; ages were secured for 1,786 animals and the chest girth measurement for 1,405 of the heifers. Age groups studied ranged from 12 to 14 mo. to 24 mo. or over. There were no significant differences in breeding efficiency between the various age groups.

There were no significant differences in the breeding efficiency of heifers grouped according to size on the basis of chest girth measurements. The estimated weight groups ranged from 301 to 400 lb. to 901 to 1,000 lb.

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A COMPARISON BETWEEN DAILY ROTATIONAL GRAZING AND CONTINUOUS GRAZING[1, 2]

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Grazing management practices with dairy cattle may be divided into two basic systems, controlled and uncontrolled. Under the uncontrolled system of management (continuous grazing) the pasture area is grazed as a single large unit for the entire grazing season. When the pasture can no longer meet the nutritive requirements of the grazing animals, it is supplemented with grain plus preserved or soiling crops.

Under the controlled system of management (rationed and rotational grazing), the pasture area is subdivided into several smaller units. These are grazed in succession, thus allowing for recovery of individual units between grazings and the harvesting of excess forage as winter feed.

Many farmers in New Zealand, using a very intensive system of rotational grazing, changing the pastures two and three times every 24 hr., have demonstrated the high productivity of this type of pasture management. McMeekan (4) reports that production of 400 lb. of butterfat per acre has been obtained using this system.

Two reasons may be advanced for increased pasture productivity under daily rotational grazing. Due to a higher intensity of grazing and the salvaging of surplus forage, a greater efficiency of utilization of available herbage is obtained. Secondly, the herbage is successively grazed while actively growing, thus assuring an adequate quantity of forage lower in lignin and of higher nutritive value than from more mature pasture forage.

This experiment has been designed to compare two extremes in pasture management, continuous and daily rotational grazing. The experiment was conducted over a complete grazing season, May 18 to August 31, 1951. Four sets of monozygotic dairy cattle twins were employed as the grazing animals, enabling a more intensive study with high significance (1, 2).

EXPERIMENTAL

Table 1 lists the animals used in the experiment. Due caution was taken to select sets in the same stage of lactation and pregnancy where these factors were involved, thereby reducing physiological as well as hereditary differences. The two steers, T94 and T95, were on different planes of nutrition prior to the start of the experiment, T95 being on a lower grain diet. This may explain some of the differences in growth rate exhibited by these two animals during the experiment.

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[2] Data presented in this paper are from a thesis submitted by A. L. Brundage to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the Master of Science Degree.

The pasture area consisted of about 10 acres of a uniform alfalfa-brome grass mixture (9 : 1) 400 by 1,000 ft. A fence divided the area down the long axis into two equal halves, and a second fence served as a lane whereby the animals managed under the daily rotational system might be admitted to the pasture at any designated point. The pasture managed under the continuous grazing system (4.8 acres) was grazed in its entirety for the whole grazing season. No clipping or harrowing was done. The pasture managed under the daily rotational system (4.6 acres) was rationed out in daily allotments. Two temporary electric fences served to separate a small strip of the pasture from the previously grazed area

TABLE 1

Experimental animals

No.	Breed (all grades)	Sex	Date of Birth	Calving date	Breeding date	Weight[a]
						(*lb.*)
T53[r]	Guernsey	F	4–23–49	4– 9–51		985
T54[c]	Guernsey	F	4–23–49	4– 2–51		990
T61[r]	Guernsey	F	4– 7–49	5– 2–51		817
T62[c]	Guernsey	F	4– 7–49	4–26–51		840
T69[r]	Holstein	F	10– 6–49		1–8–51	939
T70[c]	Holstein	F	10– 6–49		1–9–51	908
T94[c]	Holstein	M	10–27–50			500
T95[r]	Holstein	M	10–27–50			438

[a] Weight taken the day before the start of the experiment.
[r] Managed under the daily rotational system.
[c] Managed under the continuous system.

and the ungrazed area. This strip was advanced successively up the field until the previously grazed area had recovered sufficiently to permit regrazing, at which time the animals were brought back to the beginning and the ungrazed area was cut for hay.

The movability of the fence is very important in following the daily rotational system. An important part of this movability is provided by the specially designed fence posts, designed after similar ones in use in New Zealand. These consist of regular rod-shape electric fence posts, with the top part bent into the shape of a pig's tail. An old milking machine hose, slipped over this, serves as insulation, a twist allowing the wire to enter the eye, and a twist allowing the wire to fall free within the loop. This eliminates porcelain insulators and wire clips. The fence was moved as a unit to its new location each day. With experience, the two fences (400 ft.) could be moved and reerected in less than 0.5 hr. by one individual.

The four milking animals were walked to the nearby barn and milked twice a day by machine. The milk was weighed each milking and samples taken for butterfat test once a week. All the animals were weighed at the barn each Friday afternoon.

There are two factors to be considered in evaluating the two management systems: productivity of the animals and productivity of the respective pastures. Only that pasture production harvested as animal production or winter feed may

be credited to a management system. Production not harvested by the animal or man is waste resulting from the management system involved.

The method of Knott *et al.* (3) was used to calculate the nutrient requirements of the animals. No grain or roughage, except an occasional handful of coarse hay as a precaution against bloat, was fed during the experiment; therefore, it is assumed that the calculated T.D.N. requirements of the animals represent the T.D.N. production of the pastures. The fact that each experimental group was identical in genotype and performing similar physiological functions tends to reduce differences in efficiency of forage utilization, and the absence of supplemental feed tends to reduce differences in digestibility of the consumed forage. Therefore, it may be assumed that this method of estimating pasture production is a reliable means of comparison under the conditions of this experiment.

RESULTS AND DISCUSSION

Conclusions may be drawn only for the weather conditions prevailing during the experiment. These were not normal, being well below average in temperatures and above average in total and evenness of distribution of precipitation.

Definite grazing patterns were noted under each management system. The group managed under the continuous grazing system employed its own system of rotational grazing. The first few weeks of the experiment the cattle grazed down small, isolated areas of their pasture. As the experiment progressed, they continued to regraze these same areas, ignoring the remaining forage which was maturing and going to seed. Under the rainfall conditions prevailing, the regrowth on these small areas was sufficient to maintain the animals. However, visual observation indicated that this type of grazing was injurious to the pasture, for some of these areas were seriously overgrazed and other ungrazed areas were allowed to become extremely weedy.

During the early weeks of the experiment, the pasture managed under the daily rotational system was grazed very intensely, at a rate equivalent to eighty cows per acre per day. Even under this intensity of grazing, the animals were very particular in selecting the leafy material and rejecting the stems. However, when the area was regrazed, they began to select against certain small areas as well as against stems. This selectivity increased with the number of times a given area was regrazed, until at the end of the experiment they were selecting against more than 50 per cent of the area. That this was due to contamination with urine and feces, and not to differential maturity of the plants, was indicated by the observation that clipping of a small area after grazing had no effect on subsequent selectivity.

Bloat was a definite problem under both management systems and may be attributable to the predominance of alfalfa in the pasture mixture and the low roughage consumption. It is interesting to note that bloat only occurred within twin sets. The two Holstein heifers (T69 and T70) both exhibited a tendency to bloat constantly during the early weeks of the experiment. Coarse hay was given in small amounts and served to keep the symptoms from becoming too

severe, and the condition cleared up spontaneously at the end of the first 3 wk. of the experiment. The steer under the continuous system (T94) developed severe bloat symptoms in late July which lasted for over a week and then cleared up spontaneously. The steer under the daily rotational system (T95) died August 9, his death being attributed to bloat, although no previous symptoms had been observed. At this time T94 was removed from the experiment to even up the two groups. However, the performances of these two animals while on the experiment are included in the comparison of the management systems.

TABLE 2

Total milk and butterfat production and total weight gains during the experimental period

No.	Milk	Av. test	Butterfat	Weight gain
	(*lb.*)	(%)	(*lb.*)	(*lb.*)
T53[r]	2,935.1	4.58	134.31	84
T54[c]	2,835.6	4.56	129.25	62
T61[r]	1,431.9	4.84	69.29	76
T62[c]	1,357.6	4.89	66.41	97
T69[r]				132
T70[c]				125
T94[c]				148[a]
T95[r]				184[a]

[a] Removed from the experiment at the end of 11 wk.
[r] Managed under the daily rotational system.
[c] Managed under the continuous system.

There was no significant difference in total animal production resulting from either management system, as will be noted in table 2. However, if T.D.N. per

TABLE 3

Hay production from the rotationally grazed pasture

Date of cutting	Acres	Total	Lb. per acre	Total T.D.N.[a]	T.D.N. per acre
		(*lb.*)		(*lb.*)	(*lb.*)
6–12–51	2.8	7,550	2,696	3,797.6	1,356.1
7–24–51	2.8	8,975	3,205	4,514.4	1,612.1
Total	2.8	16,525	5,901	8,312.0	2,968.2

[a] Alfalfa hay, all analysis 50.3% T.D.N. (5).

TABLE 4

T.D.N. production under the two management systems

Source of T.D.N.	Total T.D.N.		T.D.N. per acre	
	Continuous	Rotational	Continuous[b]	Rotational
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Grazing	5,680.9	5,929.8 (5,649.2)[a]	1,183.5	3,138.4
Hay		8,312.0		2,968.2
Total	5,680.9	14,241.8	1,183.5	3,096.0

[a] 1.8 acres of the daily rotational pasture supplied the nutrients in parenthesis. The remainder was supplied by 6-d. grazing on that part of the pasture which also produced hay and is not included in the calculations to determine T.D.N. production per acre from grazing.
[b] 4.8 acres.

acre in the form of grazing is to be used as an evaluation of pasture productivity, it will be noted from table 4 that the daily rotational system resulted in nearly three times the production per acre of the continuous system. When T.D.N. in in the form of hay also is included, it will be noted that total T.D.N. production under the daily rotational system is almost three times that under the continuous system.

The question may be raised as to whether the decreased productivity of the continuous system is due to an excess of pasture area supplied. This is undoubt-

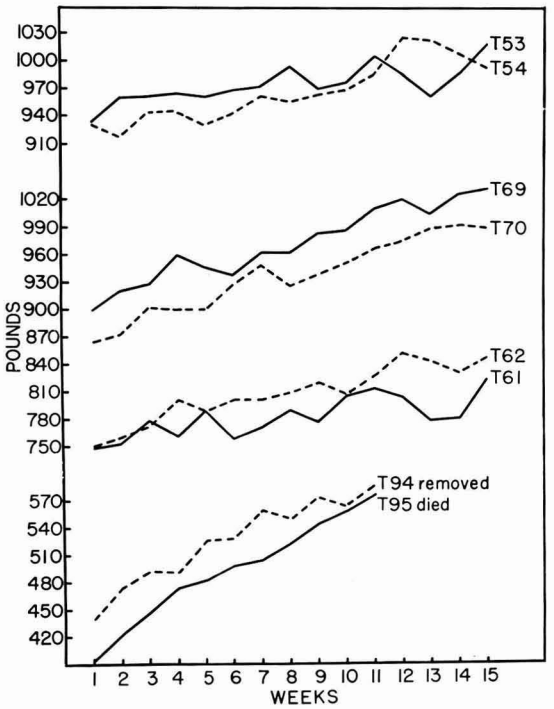

FIG. 1. Live weight of the experimental animals (4 sets of identical twins) at weekly intervals from May 18 to Aug. 31, 1951. Broken lines—continuous grazing; solid lines—daily rotational grazing.

edly a partial explanation under the weather conditions prevailing during the experiment, but not necessarily under more normal weather conditions. This illustrates an inherent disadvantage of the continuous system of management. A given pasture area must be supplied which is sufficient for the animal needs during an average pasture season, but no provision is possible for harvesting the surplus forage resulting from an exceptional growing season.

This experiment also illustates an inherent advantage of the daily rotational system. The intensity of grazing is governed by the available forage. Under very favorable growing seasons, such as the one during this experiment, less than

half of the area need be grazed, and the remainder may be cut for hay. Under more average conditions much more area would be grazed and less would be availfor hay.

Figures 1 and 2 illustrate the small weekly variation in live weight changes

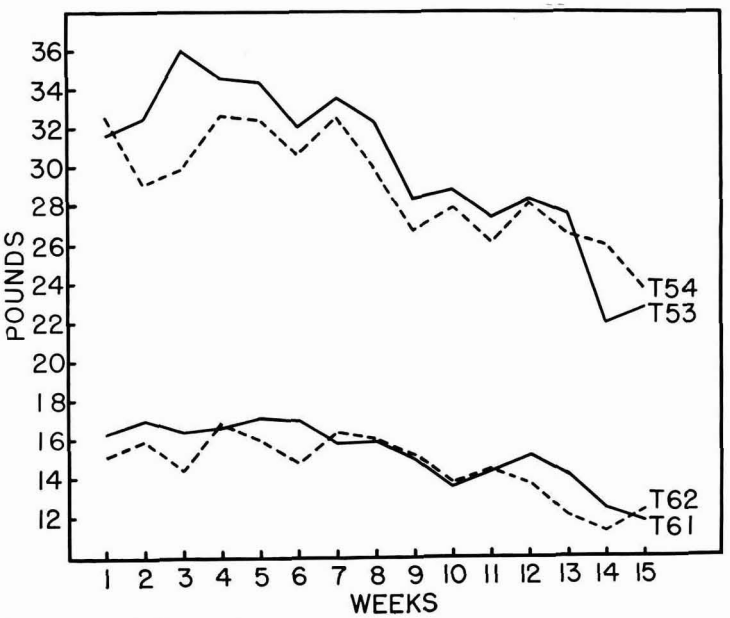

FIG. 2. Average daily 4% fat-corrected milk by weeks produced by 2 sets of identical twin Guernseys (T53 and T54 and T61 and T62) from May 18 to August 31, 1951. Broken lines—continuous grazing; solid lines—daily rotational grazing.

and milk production within sets of twins. Essentially there is little difference in animal production, either total or weekly production. However, the animals managed under the daily rotational system tended to exceed their mates in milk production and weight gains during the early weeks of the experiment. During this time the pastures were as nearly alike as at any time during the experiment, thus indicating a difference in animal production due to the management systems.

The animals under the daily rotational system declined in milk production and weight at a more rapid rate than their mates during the last third of the experiment. This decline may be attributed to faulty management of the daily rotational pasture. A second cutting of hay was taken in late July and a small part of the grazed area was clipped to ascertain the effect of clipping on subsequent selectivity of grazing. The pasture had been actively growing and it was anticipated that these areas would recover rapidly, but a cold wet August stopped growth almost completely on this area and it was necessary to maintain the animals on the previously grazed area. A slowed growth of forage here, also, plus increasing selectivity due to contamination resulted in an insufficient supply of available nutrients. That the animals were under-fed during this time is indi-

cated by the gains in weight and milk production when it was possible to put them up on the new growth on the clipped area.

This increasing selectivity of the grazing animals combined with a decreasing growth rate of the pasture plants is an important problem associated with daily rotational grazing. It necessitates careful planning to make certain that sufficient pasture forage will be available for the grazing animals when needed. This may mean saving some of the forage that ordinarily would be cut for hay for pasturing later on in the season.

The question of whether or not to employ daily rotational grazing instead of continuous grazing, considered in the light of this experiment, becomes one of efficiency of land utilization *vs.* efficiency of labor utilization. With adequate good pasture and optimum weather conditions, daily rotational grazing does not seem to increase animal productivity over that obtained with continuous grazing; but it definitely increases the number of animals which may be maintained on a given area, and provides a pasture-winter forage combination which is adaptable to changing weather conditions.

SUMMARY AND CONCLUSIONS

An experiment has been described in which four sets of identical twin dairy cattle have been used to compare daily rotational grazing with continuous grazing pasture management. The experiment was carried out over a complete grazing season from May 18 to August 31, 1951. No supplemental feed was fed.

Conclusions may be drawn only for the weather conditions prevailing during the experiment, which definitely were not normal, and for the pasture mixture studied.

There was little difference in total or weekly animal production, in the form of growth, milk and maintenance resulting from either management system.

The area grazed under the daily rotational system yielded nearly three times the production per acre as did that under the continuous system. When T.D.N. in the form of grazing and winter feed are calculated, the daily rotational system resulted in nearly three times the total T.D.N. production of the continuously grazed pasture, which was in the form of grazing only.

Animals grazing an extensive area established definite grazing patterns, overgrazing a small part of the area and allowing the remainder to mature and become weedy.

Animals managed under the daily rotational system were very selective in grazing, selecting for leafy material and against stems and contaminated material.

The necessity of close observation of both the pasture and the animals, to insure adequate intake with a minimum of wastage, in following a system of daily rotational grazing has been emphasized.

Bloat was a problem under both systems of management and has been attributed to the preponderance of alfalfa in the pasture mixture and low-roughage intake of the grazing animals.

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THE SHORT-CHAIN FATTY ACIDS OF THE PERIPHERAL BLOOD OF GOATS

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Considerable attention recently has been focused on the metabolism of short-chain fatty acids in ruminants. Volatile fatty acids now are known to be a major product of rumen bacterial fermentation (2, 4, 18). The acids thus produced evidently are absorbed directly from the rumen into the blood stream. Reid (22) and McClymont (15) have identified and measured acetic, butyric and propionic acids in the peripheral blood of sheep and cows. They reported as much as 10 mg. of volatile fatty acid per 100 ml. of blood; the majority of the acid was acetic. The blood of non-ruminants appears to contain less volatile acid (13, 17); Reid (21) found levels in dog blood as low as 1.4 mg.

The possible significance of these short-chain acids in the blood of ruminants has been suggested by several reports. Isotopic acetate has been employed by Popjak *et al.* (3, 19) to demonstrate that the mammary gland utilizes acetate to synthesize longer chain fatty acids. Manometric study (5, 6) of mammary tissue slices has revealed that under certain conditions ruminant tissue utilizes acetate more actively than non-ruminant tissue. Tyznik and Allen (24) recently have observed that the low fat content of milk produced by cows on a low-roughage ration could be increased by supplemental feeding of acetate.

The presence of these acids seems even more significant when one considers that the carbohydrate level of adult ruminant blood is somewhat lower than that of other mammals. However, the young ruminant is unusual in this respect, for at birth the glucose value may be as high as 115 mg. per 100 ml. of blood (1, 10). As the age of the animal increases, the blood glucose decreases until it reaches the adult range of 40 to 60 mg. per 100 ml. McCandless and Dye (12) have considered this change in blood glucose to be concurrent with the development of the rumen.

In this study the possibility that fatty acid levels in the blood may also reflect rumen development has been considered. The observations of McCandless and Dye have been confirmed and extended.

EXPERIMENTAL

Animals used were from the French Alpine goat herd maintained by the Dairy Science Department. Adult animals were fed on concentrates and hay. The young goats were fed milk principally, although they began to consume small amounts of hay and grain after they were about 3 wk. old. Changes in blood glucose due to ingestion of feed are minimal about 5 or 6 hr, after feeding (11).

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Also, short-chain acid levels are reported to be maximal about 4 to 6 hr. after feeding (14). Therefore, the blood samples were obtained 5 hr. after feeding.

Eighty- to 140-ml. samples of blood from adult females were obtained from the jugular vein. The young animals were stunned, the neck blood vessels were cut and the blood collected. The abdomen was opened and the stomach compartments removed as a unit. As much excess clinging tissue as possible was removed and the unit split into two parts by cutting between the reticulum and omasum. The volume of water each portion could hold was measured, then each part was dried with toweling and weighed. Volume and tissue weight were recorded as the ratio of rumen plus reticulum to omasum plus abomasum.

The short-chain fatty acids were estimated and identified by means of a partition chromatography method which included techniques described by Peterson and Johnson (16) and Ramsey and Patterson (20). A Celite-water-benzene slurry containing brom-cresol green to locate the position of the acids was packed tightly in a glass tube (a 25-ml. burette cut off so that 10 cm. of tube remained above the stopcock) until the column was about 3 cm. long. The rate of percolation was controlled by pressure from a cylinder of nitrogen.

The blood serum was diluted, deproteinized with zinc hydroxide (23), acidified and then distilled as described by Friedeman (7). The distillate was titrated with 0.01 *N* NaOH to a phenolpthalein end point under a nitrogen atmosphere. The titration values obtained on these distillates were always irregular. Only a small portion of the value actually was found to be due to fatty acids as determined by the chromatographic procedure. This irregularity and the high titration of the distillates were presumed to be due to the presence of CO_2 which could not be removed completely. Therefore, to obtain an acceptable estimate of total volatile fatty acids, chromatographic measure was necessary.

The neutralized solution was concentrated to a small volume and then evaporated to dryness. The dry salts were acidified, extracted with benzene and transferred to the top of the Celite column. The removal of all acids with three or more carbons from the column by continuous percolation of benzene was followed by elution of acetic acid with a solution of 25 per cent butanol in chloroform (CB-25). The acid fractions collected were titrated with standard 0.01 *N* sodium ethylate under a nitrogen atmosphere to a phenolthalein end point. With known solutions of acetic acid, it was found that organic-solvent solutions gave lower titration values than water solutions. Thus, correction factors of 1.107 for benzene solutions and 1.085 for CB-25 solutions were used to calculate amounts of acid present.

Glucose was determined by a modification of the micro-method of Folin-Malmros, used routinely in this laboratory (9).

RESULTS

The rumen of the goat 6 days old (table 1) was extremely small, undeveloped and contained virtually no solid material. Goat 2, which was 26 days old, had some intact hay in the rumen and limited fermentation may have occurred. Some fermentation seemed evident in the rumens of all other animals. The

TABLE 1

Rumen development and changes in blood glucose in goats

Goat	Age	Blood glucose	Volume ratio[a]	Tissue weight ratio[a]
	(*d.*)	(*mg./100 ml.*)		
1	6	113	0.32	0.60
3	31	106	2.17	1.24
2	26	103	0.88	1.34
4	46	90	1.67	1.56
6	66	88	2.09	1.58
5	52	73	2.22	1.74

[a] Ratios of rumen and reticulum to omasum and abomasum.

ratios of the volume and weight of tissue of rumen and reticulum to omasum and abomasum served as an approximate comparison of development of the two major portions, *i.e.*, rumen and abomasum. As would be expected, there was considerable difference among individuals as to actual size; however, both ratios emphasize the rapid development of the rumen in comparison to the abomasum. Blood glucose values (table 1) were similar to those previously reported in the literature. At 6 days the value was 113 mg. per 100 ml.; the blood glucose decreased and the ratios of rumen to abomasum increased as the animal became older.

The results of the fatty acid analysis are presented in table 2. In the chromatographic method used, all acids with four or more carbons were eluted from the column in one fraction with no possible separation into single acid groups. Since this particular fraction was small and probably was mostly

TABLE 2

Short-chain fatty acids in blood of goats

Age	Fatty acid concentration (μM/100 ml.)					Per cent of acetic acid of total acid (*molar*)
	Acetic acid	Propionic acid	Propionic acid[c]	Butyric acid[b]	Total volatile acid[a]	
(*d.*)						
6	37.4	3.9	6.2	2.3	43.6	85.8
26	50.6	6.2	9.9	3.7	60.5	83.7
31	37.8	6.8	11.8	5.0	49.6	76.2
52	47.7	5.0	8.4	3.4	56.1	85.0
66	51.4	5.0	9.3	4.3	60.7	84.6
Mean	45.0	5.4	9.1	3.7	54.1	83.8
Adult	71.7	.....	21.8	.....	93.5	76.7
"	64.9	.....	29.7	.....	94.6	68.5
"	64.4	.....	17.1	.....	81.5	79.0
"	48.8	.....	17.3	.....	66.1	73.8
"	68.1	8.0	18.9	10.9	87.0	78.3
Mean	63.6		21.0		84.6	75.3

[a] All volatile acids with more than 1 carbon atom.
[b] All volatile acids with more than 3 carbon atoms calculated as butyric acid.
[c] All volatile acids with more than 2 carbon atoms calculated as propionic acid.

butyric acid, it is reported as butyric acid. In some of the analyses, no separation was observed between the propionic and butyric acid fractions. In these cases, the fraction of acids with three or more carbons was calculated as propionic acid and tabulated in a separate column.

Acetic acid was found (table 2) in the range of 48–72 μM per 100 ml. in the blood of the adult goats and in the range of 37–51 μM in the blood of the young. There were 17–30 μM of volatile acids with more than two carbons in the adult blood and 6–12 μM in the young blood. In milligrams per 100 ml., the average amount of acids with more than one carbon was 5.4 mg. in adult venous serum and 3.4 mg. in young serum. Thus, the adult blood contained appreciably larger amounts of all acids than the blood of the kids. Propionic and butyric acid fractions showed a greater percentage increase than acetic acid.

DISCUSSION

The levels of volatile fatty acids found in the blood of adult goats agree with the values reported to be present in the venous blood of cattle and sheep. However, there appears to be some difference in the type of acid comprising the volatile fraction. In the adult goat, acetic acid constitutes about 75 per cent of the volatile acids as calculated from the molar per cent of the acids liberated from the column, while in cattle and sheep acetic acid accounts for more than 85 per cent of this fraction. This may be a species difference or a difference in analytical procedure.

It should be emphasized that titration of the distillate reflected acid values in excess of the total combined amounts of acetic, propionic and butyric acids in the sample. This became evident on comparison of this titration value with the sum of the acids recovered from the sample on chromatography. A small amount of acid(s) was observed to migrate more slowly than acetic acid, as was indicated when some of the columns were treated with butanol after the acetic acid had been recovered with CB-25. A chemical determination (8) indicated the possible presence of formic acid in this fraction. Some of this acid may have been a breakdown product of the carbohydrates in the blood which were produced during distillation; however, a small amount was present in the blood.

Although the rumen generally is conceded to be the source of blood volatile acids in ruminants, a considerable amount is observed in the blood of young goats. The source of this acid is probably intestinal bacteria or internal metabolic activity. However, it is of interest that these levels in the young goat are still somewhat higher than those reported by Reid for dogs (21).

The development of the rumen is concurrent with a transition period in the metabolic patterns of the young animal. The decrease in blood glucose as the rumen becomes important from the standpoint of volume and weight of tissue is a reflection of this change. A further reflection of this transition is the higher levels of volatile acids in the adult animals. Although the data established a difference between young and adult levels of volatile acids, they do not definitely indicate whether the volatile acids increase gradually with rumen development or if they are influenced by a particular type of fermentation. Since the tissues

may utilize these acids, lower levels should be expected in venous blood. In this study, venous blood from the adult is compared with a mixture of venous and arterial blood from the young; this may lessen the observed differences between young and adult animals.

As to its energy source, the young ruminant then is similar to the non-ruminant. Monosaccharides probably are absorbed from the digestive tract and account for the high levels of blood glucose. When the rumen develops and begins to function, the short chain acids may become a major product of the digestive tract and may constitute an important energy source for the animal. This accounts for the rise in acid level and the decrease in blood glucose. Reid (21) has discussed the probability that the source of the blood glucose is the propionic acid formed in the rumen.

SUMMARY

Measureable amounts of acetic, propionic and butyric acids were identified in the peripheral blood of goats. Small amounts of other volatile acids also were present. There were 2.7 to 3.8 mg. of total volatile acids per 100 ml. in the blood of the young goats and 4.2 to 6.1 mg. per 100 ml. in the blood of the adult animals. About 75 per cent (molar basis) of the adult levels of volatile acids was acetic acid.

Concurrent with the development of the rumen, the glucose level of the blood decreased, while the short-chain fatty acid level increased.

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THE PREFERENTIAL UTILIZATION BY BULL SPERMATOZOA OF GLUCOSE AS COMPARED TO FRUCTOSE[1]

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In studies on the rate of fructolysis by bull spermatozoa, as a possible indication of inherent fertility level of the semen under consideration, it was noted in this laboratory that the utilization of fructose was always depressed in the presence of the yolk-citrate-sulfanilamide diluter. Mann (5), who advocated fructolysis determinations for such purposes, incubated whole bull semen in a Ringer's-phosphate buffer at 38° C. He reported fructose utilization of from 1.5 to 2.0 mg. per 10^8 spermatozoa per hour for normal semen samples. Our average value of fructose utilization by 42 comparable semen samples in yolk-citrate-sulfanilamide (2) at 46.5° C. was 0.11 mg. per 10^8 spermatozoa per hour. Inasmuch as the buffer and other incubation conditions were somewhat different in our study, an investigation was made to determine the actual cause of the difference in fructolysis rate. It is the purpose of this paper to present those results.

EXPERIMENTAL PROCEDURE

The incubation of diluted semen was carried out in narrow test tubes, making the condition essentially anaerobic or in Warburg vessels under an atmosphere of nitrogen. The routine dilution rate was one part of semen to four parts of added diluent. Total reducing substances (TRS) were determined by the method outlined by Nelson (8), fructose by the method of Roe (9) and lactic acid by the method of Barker and Summerson (1). Glucose, where pertinent, was calculated as the difference between the TRS and the fructose analysis. Purity of the sugars added was tested polarimetrically. The specific rotation obtained for glucose was 52.75° and for fructose −88.0°. The purity of these commercial products was such that no further purification seemed necessary.

RESULTS

The rate of fructolysis of whole semen when incubated at 46.5° C. in a calcium-free Ringer's-phosphate medium (3) was compared to that in a yolk-citrate-sulfanilamide diluent which contained additional carbohydrates. One-half of each of five semen samples was incubated in each of the two diluents in test tubes. The results presented in table 1 establish the fact that a real difference existed in fructose utilization between the two diluents during incubation at 46.5° C., the differences noted being statistically highly significant ($P = \text{or} <$

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[1] The data for this paper are from a thesis presented by the senior author to the Graduate College, University of Illinois, in partial completion of the requirements for the degree of Master of Science in Dairy Science. June, 1951.

TABLE 1

Glucose and fructose utilization and lactic acid production by bull semen. (Incubations were made at 46.5° C. for 60 min. in narrow test tubes. Average values are given for five incubations. Bull semen diluted 1 : 4 with the appropriate buffer.)

Diluter	TRS* Initial loss		Fructose Initial loss		Glucose Initial loss		Lactic acid Gain
				(*mg./10⁸ cells*)			
Ringer's-phosphate	0.41	0.19	0.51	0.23	− 0.10	− 0.04	0.21
Yolk-citrate-sulfanilamide	0.73	0.23	0.54	0.04	0.19	0.18	0.25

* TRS = Total reducing substances

0.01). Also, they show that at this relatively high incubation temperature the main metabolic channel is a glycolytic one, lactic acid being the primary end-product of the carbohydrate used. The discrepancies observed between TRS and fructose determinations in the Ringer's-phosphate diluent are unexplained, but seem not to vitiate the conclusion that a real difference in fructose utilization in the two diluents occurs at 46.5° C. The results suggested that a source of carbohydrate furnished by egg yolk was used in preference to fructose.

Thus, a second 46.5° C. incubation experiment involving three semen samples was conducted with the Ringer's-phosphate as the buffer, to which (a) equal parts of fresh egg yolk, (b) fructose and (c) glucose were added. The latter two were added in amounts approximately equal to the level of TRS in the amount of yolk added in (a). The results are shown in table 2. Essentially

TABLE 2

Glucose and fructose utilization by bull semen incubated in Ringer's-phosphate buffers containing either egg yolk or added carbohydrate. (Incubations were made at 46.5° C. for 60 min. in narrow test tubes. Average values are given for three incubations. Bull semen diluted 1: 4 with the appropriate buffer.)

Addition	TRS		Fructose		Glucose	
	Initial loss		Initial loss		Initial loss	
			(*mg./10⁸ cells*)			
Egg Yolk	0.73	0.22	0.43	− 0.01	0.30	0.23
Glucose	0.68	0.20	0.43	0.05	0.25	0.15
Fructose	0.69	0.20	0.65	0.22	0.04	− 0.02

the same picture was obtained from the added egg yolk with the Ringer's-phosphate buffer as when the sodium citrate buffer was used earlier. Practically no fructolysis resulted, while TRS were utilized by the spermatozoa as in the earlier experiments, which led to the conclusion that the type of buffer was not the main item causing the depression in fructose utilization.

In order to study further the effect of glucose under conditions in which the level of glucose or of fructose could be controlled, and to test whether or not the phenomenon occurred at normal body temperature, the fructose normally found in semen was removed by washing the spermatozoa two times in the Ringer's-phosphate and diluting to the original volume with buffer. To separate quantities of the Ringer's-phosphate solution equal quantities of glucose, of

fructose and of a mixture of equal parts of each were added. Equal amounts of washed spermatozoa from each of four semen samples were added to the solutions and incubated in a Warburg flask under nitrogen for 1 hr. at a temperature of 37° C. The average level of sugar added to each flask was 257.2 mg. per 100 ml. of sperm suspension.[2] In the mixture containing both glucose and fructose, an average of 83.5 mg. per 100 ml. of glucose was utilized as compared to but 0.5 mg. per 100 ml. of fructose. An average of 107.0 mg. per 100 ml. of substrate was utilized in the diluent containing only glucose, while in the fructose-containing flask, an average of 78.2 mg. per 100 ml. was utilized. These results pointed conclusively to the fact that under anaerobic conditions at 37° C. spermatozoa in the presence of equal quantities of both glucose and fructose preferentially metabolized the glucose. Thus, the results were not qualitatively different for the two different incubation temperatures employed in these series of experiments, 46.5 or 37° C.

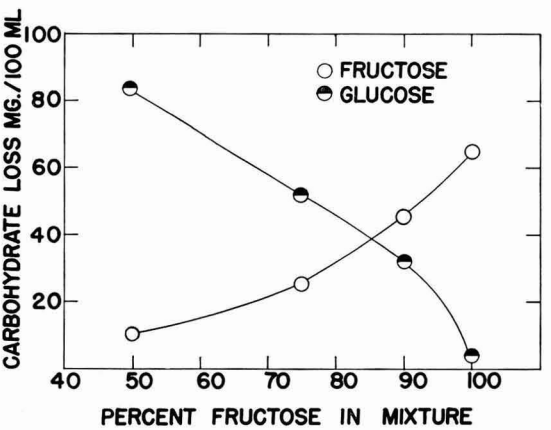

FIG. 1. Glucose and fructose utilization by washed spermatozoa from various mixtures of the carbohydrates in a Ringer's-phosphate medium. Average values of 6 semen samples.

For the determination of the approximate concentration of the two substrates at which utilization would be essentially equal, the following experiment was designed. Twice-washed spermatozoa from six semen samples were incubated at 37° C. under nitrogen in a Warburg flask in Ringer's-phosphate buffer containing varying proportions of fructose to glucose, including 1:1, 3:1 and 9:1 parts of fructose to glucose and one flask containing only fructose. These monosaccharides were added to a total level of approximately 0.67 mg. of TRS per 10^8 cells in all flasks in each of the six replications, an amount sufficient to meet the total substrate need of the spermatozoa. The results presented in figure 1 show that under the conditions of this experiment, and with the total supply of available substrate used, the two lines cross at an extrapolated ratio of 85:15 fructose to glucose, indicating equal rates of utilization at this point.

[2] In this experiment sperm counts were not made in all samples after washing and, therefore, the results are given in mg. of glucose per 100 ml. of sperm suspension.

DISCUSSION

The yolk from hen's eggs has been reported (7, 10, 11) to contain glucose. The difference noted in fructolysis rates in the two buffers is undoubtedly due to the glucose content of the added egg yolk. The studies reported in this paper confirm the observation of Mann (6) that spermatozoa in their own seminal plasma, or free from it under anaerobic conditions (preferentially), utilize glucose when it is available.

The studies by Wiebelhaus and Lardy (13) and by Slein *et al.* (12) in which hexokinases from yeast and from animal tissues were shown to have a much higher affinity for glucose than for fructose might offer a possible explanation for the results obtained with bull spermatozoa. It is postulated that bull spermatozoa contain an enzyme or enzymes which act similarly to these other hexokinases. This hypothesis, however, can only be tested by isolation or purification of the enzyme or enzymes from the bull spermatozoa.

Whether the preferential glucose utilization by bull spermatozoa has any relation to the metabolism of the spermatozoa in the female genital tract is not known but has further to be investigated.

SUMMARY

Egg yolk has a sparing effect on fructose utilization by bull spermatozoa during anaerobic incubation at 37 or 46.5° C.

This sparing effect can be explained by the occurrence of glucose in egg yolk and the utilization of glucose in preference to fructose by bull spermatozoa.

At a ratio of 85 parts of fructose to 15 parts of glucose, an equal utilization of fructose and glucose by washed spermatozoa may be expected during incubation at 37° C. under nitrogen, if the initial level of TRS is approximately 0.67 mg. per 10^8 cells.

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VOLUME XXXV JULY, 1952 NUMBER 7

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BUTTER

O. F. HUNZIKER, SECTION EDITOR

360. Wrapping machine for butter and the like. H. R. COON, SR. AND E. J. RAPP (assignors to Lynch Pkg. Machinery Corp.). U. S. Patent 2,592,793. 1 claim. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 758. 1952.

Details are given covering the construction of a motor-driven machine for wrapping prints of butter, margarine, etc. R. Whitaker

361. Infrared ray butter softener. E. F. HUBACKER (assignor to Borg-Warner Corp.). U. S. Patent 2,594,023. 5 claims. Apr. 22, 1952. Official Gaz. U. S. Pat. Office, **657,** 4: 1176. 1952.

Butter, within a household refrigerator, is maintained at a temperature suitable for spreading by a thermostatically controlled infrared lamp. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

362. Factors in the rise of yogurt and other cultured milks. C. B. LANE, Breakstone Bros., Inc., Walton, N. Y. Food Eng., **24,** 3: 59, 60, 161. Mar., 1952.

The popularity of yogurt and cultured buttermilk has been increasing as a result of the attitude of diet-conscious Americans. Although there may be a question as to the curative value of these products, many people now buy them as a food rather than a health treatment. Yogurt contains lactobacilli, probably *L. bulgaricus,* and a strain of *S. thermophilus.* Commercial yogurt is not easy to make correctly. Cultured buttermilk contains *S. lactis* with *S. citrovorous* and *S. paracitrovorous.* Procedures are given for the preparation of yogurt, acidophilus milk and other cultured products. T. J. Claydon

363. Growth of pigs given skimmilk soured with nisin-producing streptococci. R. W. BARBER, R. BRAUDE AND A. HIRSCH. Nature, **169,** 4292: 200. 1952.

Pigs fattened for 126 d. on a daily diet containing 4–6.5 lb. of skimmilk soured with *Streptococcus lactis* failed to grow any better than a control group fed unsoured skimmilk. Cultured skimmilk contains 100–120 units/ml. of nisin, the antibiotic produced by *S. lactis.* R. Whitaker

364. Preservation of foodstuffs. L. E. BORST. Application 552,558. Apr. 29, 1952. Official Gaz. U. S. Pat. Office, **657,** 5: 1582. 1952.

Food in containers is conveyed through an air-cooled, graphite-moderated natural uranium reactor to produce sterility. The product is stored after treatment to allow decay of any radioactive material resulting from the use of the short-lived fission products. R. Whitaker

365. Method for use in the preservation of eggs. A. NAPPER. U. S. Patent 2,595,808. 2 claims. May 6, 1952. Official Gaz. U. S. Pat. Office, **658,** 1: 221. 1952.

A solution of 3–10% Ca and Na caseinate and $CaCO_3$ is used to seal the porous shells of eggs. The submerged eggs first are subjected to a vacuum to remove the air, then to a slight pressure, which causes the sealing solution to penetrate the shells. R. Whitaker

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

366. Reagents for iodometric determination of peroxides in fats. L. HARTMAN AND M. D. L. WHITE, Dept of Scientific and Ind. Research, Wellington, New Zealand. Analyt. Chem., **24,** 3: 527–529. 1952.

An investigation was carried out to develop an improved reagent for the iodometric determination of peroxides in fats that would not give high blanks or react with some of the liberated iodine. All reagents were found to be subject to error due to liberation of iodine by atmospheric oxygen. The only advantage presented by a good reagent was in the elimination of the need for blanks. The use of an approximately 10% solution of citric acid in a mixture of tert-butyl alcohol and carbon tetrachloride gave results that were in good agreement with the acetic acid-carbon tetrachloride mixture generally used. The new reagent could be used without making a blank titration. B. H. Webb

367. The detection of enzymes by the chromatographic brush method. II. L. ZECHMEISTER AND M. ROHDEWALD, Calif. Inst. of Technol., Pasadena. Enzymologia, **15,** 3: 109–114. 1952.

Techniques used were summarized previously in the abs. (no. 149) of the 1st paper of the series. Methods have been developed for detection of an additional group of enzymes including α-glucosi-

dase, α-galactosidase, β-galactosidase, β-glucuronidase, phosphorylase, hydroxynitrilase, and urease. These enzymes may be detected alone or simultaneously when occurring in mixtures of 2–5 enzymes of selected types. J. J. Jezeski

368. The amperometric titration of plasma chloride and urine chloride. C. W. Carr, Univ. of Minn., Minneapolis. Arch. Biochem. Biophys., **34,** 2: 299–304. Dec., 1951.

An adaptation of the amperometric titration procedure of Laitenen *et al.* (*Ind. Eng. Chem.*, Anal. Ed., **18:** 355. 1946.) to chloride analysis of bovine serum and urine achieved results within 1% of those obtained with the methods of Volhard and Schales and Schales. To determine serum chloride, 1 ml. of sample was diluted with 21.5 ml. water, 2.5 ml. conc. HNO_3 and 25 ml. acetone; titration with 0.01 *N* $AgNO_3$ employed the rotating platinum electrode as the indicator electrode. Samples high in protein, such as whole blood and muscle tissue, foul the platinum wire; however, amperometric titration of the ash of such samples is suggested. H. J. Peppler

369. Moisture teller. H. W. Dietert and A. L. Graham (assignors to Harry W. Dietert Co.). U. S. Patent 2,594,743. 1 claim. Apr. 29, 1952. Official Gaz. Pat. Office, **657,** 5: 1452. 1952.

Details are given for the construction of the Dietert moisture tester, a device for determining the total solids of various products, including dairy products. R. Whitaker

370. Recovery of protein from whey. M. E. Hull (assignor to Armour and Co.). U. S. Patent 2,595,459. 4 claims. May 6, 1952. Official Gaz. U. S. Pat. Office, **658,** 1: 127. 1952.

Whey first is neutralized to an acidity of 0.0–0.06%, then heated to 180–212° F. with agitation. The protein is ppted. by adjusting the pH to 4.1–4.5, and then removed from the whey.

R. Whitaker

371. Process for the preparation of lysozyme from ass milk. D. M. Polo and A. Guillermo. U. S. Patent 2,590,121. 5 claims. Mar. 25, 1952. Official Gaz. U. S. Pat. Office, **656,** 4: 996. 1952.

Lysozyme, precipitated from ass milk by acetone, is purified by a series of refining extractions and reprecipitations and finally dried at 10° C. under vacuum desiccation over P_2O_5.

R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

372. Refrigerated liquid storage tank. F. J. Zamboni. U. S. Patent 2,594,603. 10 claims. Apr. 29, 1952. Official Gaz. U. S. Pat. Office, **657,** 5: 1411. 1952.

A cold wall storage tank has a circulating liquid in the jacket and is cooled by a series of separately controlled refrigerated cooling coils located within the jacket. R. Whitaker

373. Centrifuge for separating cream from cold milk products. G. J. Strezynski (assignor to the De Laval Separator Co.). U. S. Patent 2,593,934. 17 claims. Apr. 22, 1952. Official Gaz. U. S. Pat. Office, **657,** 4: 1150. 1952.

Construction details are given for a milk separator bowl for handling cold milk.

R. Whitaker

374. Apparatus for removing the product film from a revolving drum surface. C. O. Lavett (assignor to Blaw-Knox Co.). U. S. Patent 2,592,914. 8 claims. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 791. 1952.

A series of individually controlled knives, and means of adjusting same are described for removing products like milk powder from the surface of drum driers. R. Whitaker

375. Device for stabilizing and homogenizing liquid mixtures. S. H. B. Zachariassen (assignor to Aktiebolaget Separator). U. S. Patent 2,595,376. 3 claims. May 6, 1952. Official Gaz. U. S. Pat Office, **658,** 1: 104. 1952.

Details are given for a homogenizer of the centrifugal-bowl type. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSON, SECTION EDITOR

376. Whats ahead for the dairy industry. P. H. Tracy, Univ. of Ill., Urbana. Food Eng., **24,** 4: 80–83. Apr., 1952.

The need for more economical production coupled with the competition from substitute products will induce changes in the dairy industry. The trends pointed out by the author are toward fewer cows, shifts in dairying locales, drop in milk and cream consumption, a smaller number of plants, greater sales through stores, a rise in vending-machine sales, standardization of sanitary rules, wider interest in low-fat foods, more substitute products, a switch to higher quality butter, centralization of operations, a swing to continuous processing, automatic packing of products, washing of pipelines in-place, tank-truck pick up from farms and better labor relations.

T. J. Claydon

377. Class II milk—an important but neglected price. E. E. Vial, Milk Dealers Assn. Metrop. New York, Inc. Am. Dairy Prod. Mfg. Rev., **14,** 3: 2–4, 6, 18–20. Mar., 1952.

In the New York area, fluid cream (class II milk) is the 2nd most important single outlet for pool milk, but the volume has decreased 30% in the past decade and only 10% of the pool milk is so utilized. Pricing of this class has received little consideration in federal order hearings. The change in price schedule effective May, 1940, increased the Class II price in relation to butter especially when butter prices are in a high range as at present. The arbitrary limits on the source of cream supply, plus the federal order, result in relatively high Class II prices in the New York area. Prices are considerably higher than in Boston and Philadelphia markets. Many supporting data and charts are presented.

T. J. Claydon

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

378. Fertilizing capacity of bull spermatozoa after freezing at – 79° C. C. POLGE. Nature, **169,** 4302: 626. 1952.

Semen, on collection, was diluted with an equal volume of yolk-citrate buffer at +28° C. and cooled to +5° C. in 4 hr. It again was diluted with an equal volume of citrate buffer containing 20% glycerol. After standing over night at +5° C. the temperature was lowered to −79° C. in 45 min. and maintained for 8 d. Pregnancies were produced in 79% of the 38 cows inseminated with the thawed material. R. Whitaker

379. The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattel. D. ANDERSON, R. E. BILLINGHAM, G. H. LAMPKIN AND P. B. MEDAWAR, Univ. of Birmingham, and The Animal Breeding and Genetics Research Org., Edinburg. Heredity, **5,** 3: 379–397. 1951.

Skin was interchanged between cattle that were unrelated, full siblings, dizygotic twins of unlike sex and between those of the same sex, diagnosed phenotypically either as dizygotic or as monozygotic. Grafts between both monozygotic and dizygotic twins were effectual, whereas skin grafts between the cattle in other groups given above were destroyed rapidly. The tolerance of dizygotic twins to each others skin was not always complete. It was suggested that the degree of tolerance may be the same as the degree of similarity of their antigenic types. L. O. Gilmore

380. Monozygotic bovine quadruplets. H. P. DONALD, W. S. BIGGAR AND D. M. LOGAN, Animal Breeding and Genetics Research Org., Edinburgh. Heredity, **5:** 135–142. 1951.

These calves were considered by the authors to be the 1st set of monozygotic calves to be diagnosed. The conclusion of monozygosity was based on the similarities between calves in 32 observations in 11 different categories of characteristics. The dam, and supposedly the sire, were British Friesian. The calves were purchased but some died before further studies could be made.

The theoretical explanation for the frequency of occurrence of monozygotic quadruplets based on Hellins' law for human data and twinning rate in Swedish cattle is given. A table of bovine quadruplets, quintuplets and sextuplets from cases in the literature is given. L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

381. Barn gutter cleaner. H. O. PETRASKE (assignor to James Mfg. Co.). U. S. Patent 2,593,340. 1 claim. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 908. 1952.

A bar, laid in the corner of the gutter, is given a reciprocating motion by a 2-stroke hydraulic ram. At intervals, spaced less than the distance of the stroke, a series of hinged paddles are attached to the bar. The paddles swing outward on the forward stroke and push the litter, then fold inward on the back stroke, thus gradually moving the litter to the end of the gutter. Means are provided to keep the litter from getting into the hinge. R. Whitaker

382. Milking plant. H. S. Palmer. U. S. Patent 2,593,597. 3 claims. Apr. 22, 1952. Official Gaz. U. S. Pat. Office, **657,** 4: 1059. 1952.

An aisle for the milking attendant is provided at a lower level, between 2 stalls for milking cows. R. Whitaker

383. Animal restraining device. L. E. HELDENBRAND. U. S. Patent 2,593,559. 2 claims. Apr. 22, 1952. Official Gaz. U. S. Pat. Office, **657,** 4: 1049. 1952.

Details are given for the construction of a device for rigidly holding an animal, such as a cow, steer, etc., for branding and transporting, and while giving the animal medical attention. R. Whitaker

384. Bull lead with quick release. D. O. WENDT. U. S. Patent 2,595,432. 1 claim. May 6, 1952. Official Gaz. U. S. Pat. Office, **658,** 1: 119. 1952.

A device for leading cattle consists of a pivoted member similar to pliers in operation. A rope is attached to 1 handle and threaded through a spring attachment in the other handle which tightens the device on the animal's nose the more the rope is pulled. R. Whitaker

385. Nose tattooing device for cattle. C. S. CRANE AND R. A. DOVE. U. S. Patent 2,593,110. 3 claims. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 845. 1952.

A device for marking noses of cattle and other animals for identification purposes is described. R. Whitaker

386. Dilator for clogged teats. V. GARIEPY. U. S. Patent 2,592,800. 1 claim. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 760. 1952.

An instrument is decribed for introducing medication into teats of cows, etc. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

387. Looking back on a quarter of a century of advancement in ice cream research. C. KOERVER, Borden's Pioneer Ice Cream Div., Brooklyn, N. Y. Ice Cream Trade J., **43,** 4: 34, 111. Apr., 1952.

During this period, paper cans have largely replaced metal, many new ice cream stabilizers have been developed and, where available, liquid sugar has resulted in large saving in labor and handling costs. Stabilization of fruit and improvement of chocolate flavor are other problems studied by Dr. Koerver. W. H. Martin

388. Case history demonstrates four big modernization benefits. A. V. GEMMIL. Food Eng., **24,** 3: 49–53, 214, 215. Mar., 1952.

Modernization of Borden's ice cream plant at Gouverneur, N. Y., resulted in greater capacity, smaller losses, reduced expenses and better con-

centrates. These advantages were achieved by installation of some new equipment and slight modifications in design, but without excessive changes in the plant. Details of modernization are given. T. J. Claydon

389. New low-fat high-protein package. Anonymous. Ice Cream Trade J., **48,** 3: 28, 58. Mar., 1952.

"Chek" is the name of a new low-fat, high-protein frozen ice milk which has been introduced by members of the Quality Chekd Dairy Products Assoc. The product is sold in qt., fifth-gal. and pt. cartons in vanilla, chocolate and strawberry flavors. W. H. Martin

390. Hood's reports on its insulated overwrapped pint. Anonymous. Ice Cream Trade J., **43,** 4: 26, 27, 102. Apr., 1952.

On July 25, Hood's brought the insulated overwrapped package into the metropolitan Boston area for the 1st time after marketing tests in various districts under the Polar Sealed name. As Hood's expanded the market for this insulated overwrapped package, the regular type of pt. container was being withdrawn in each of the districts.

Sales experience varied in different areas during the 10-d. introductory period when the Polar Sealed Pint was being sold for 29¢. The increases were anywhere from 150–300% plus over last year. After wholesale and retail prices went back to normal, during an 8-wk. period there was an average plus in all areas of 35%, as compared with last year. Regular pint is being produced only in the Polar Sealed overwrap.

Consumer acceptance is due to retention of flavor and texture because of the sealing-in process and shopping convenience to consumers. Hood's believe larger ice cream companies will adopt a similar method of protecting their ice cream quality until the product is consumed by customers. W. H. Martin

391. Analysis of the industry's volume in bulk, packages, specialties and cups. Anonymous. Ice Cream Trade J., **48,** 3: 22–24, 118–119. Mar., 1952.

An analysis of ice cream sales made by IAICM shows many changes in types of products and container sizes during the past 10 yr. Volume of bulk ice cream sold has declined from 63.11% of sales to 46.15; packaged ice cream including pt., qt., 0.5-gal. and gal. increased from 20.07 to 29.81%, cups increased from 5.47 to 6.48% and frozen specialties were up from 11.35 to 17.56% during the period 1941–1950.

The survey was based on reports from 415 ice cream manufacturers with a sale of 163,501,809 gal. W. H. Martin

392. Consumer research can aid in ice cream merchandising. F. A. Babione, Penn. State College, State College, Pa. Ice Cream Trade J., **43,** 3: 90–91, 112. Mar., 1952.

Methods used in ice cream consumer research, including taste test studies, consumer interviews, dealer interviews, store observation, sales tests and records, are discussed. W. H. Martin

393. Two-decker dairy self-service refrigerator. R. E. Pabst (assignor to E. Friedrich, Inc.). U. S. Patent 2,594,066. 2 claims. Apr. 22, 1952. Official Gaz. U. S. Pat. Office, **657,** 4: 1188. 1952.

A refrigerated self-service display case or cabinet for dairy products is described. Available storage space and display area are extended by having a trough-like shelf, backed by a refrigerated cooling coil, set back and above the usual storage area. R. Whitaker

394. How and what to sell through vending machines. M. Wainer, Pony Boy Ice Cream Co., Lancaster, Pa. Ice Cream Trade J., **48,** 3: 52, 53, 108. Mar., 1952.

Successful selling of ice cream through vending machines requires a variety of items and use of proper locations. Ways of securing locations are discussed. Proper equipment, strategic location and proper maintenance and service are important factors in successful vending machine operation.

A change of item at least once a week and use of illuminated signs are effective in increasing sales; attractive trucks and uniformed drivers and servicemen help advertise the product. On each machine is a decal to show the owners' name and day and night number for service calls.

W. H. Martin

395. Ice cream scoop. M. C. Merger. U. S. Patent 2,592,720. 2 claims. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 738. 1952.

A hand-operated scoop for forming ice cream into pie-shaped portions is described.

R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

396. National program for interstate milk shipments. L. A. Scheele and H. G. Hanson, Pub. Health Service, F.S.A. Pub. Health Repts., **67,** 3: 260–267. 1952.

The authors present the views of the Public Health Service on a national program for interstate milk shipments. Background information on the responsibilities of this organization in the field of milk sanitation is outlined first, followed by what are considered to be the problems involved in shipping milk in interstate commerce. The PHS believes that 1 important step toward a successful interstate milk shipment program would be the establishment of uniform health and sanitation regulations for the shipping areas and reciprocity in the inspection of milk.

Members of state health departments, dairy industry, agriculture departments and the PHS held conferences and formulated a plan and procedure for interstate milk shipping. The participation of the PHS is specifically requested in certain parts of this program as follows: (a) State of origin is to make milk sanitation ratings of the

milk shed following procedures developed by the PHS and the ratings reported to the PHS for certification. (b) Spot checks are to be made of the inspection, laboratory and rating procedures used by each state. (c) The PHS is to publish lists of interstate shippers as rated by shipping states. (d) The PHS, when requested, is to assist states in carrying out their milk control program. (e) The milk ordinance and code recommended by PHS is to be used as a basis for rating interstate milk suppliers.

D. D. Deane

397. New advance in canned fresh milk. C. R. Havighorst. Food Eng., **24,** 3: 39. Mar., 1952.

The procedure developed by Golden State Co. utilizes conventional methods of handling and transporting milk from farm to plant. Special techniques have been devised for pre-heating, homogenizing, HTST sterilizing, holding and cooling. The product is canned aseptically in the Martin unit. A pilot plant is in operation.

T. J. Claydon

398. Apparatus for manufacturing whipped cream. N. J. Pfeiffer and F. F. Suellentrop (assignors to Lemay Machine Co.). U. S. Patent 2,594,492. 12 claims. Apr. 29, 1952. Official Gaz. U. S. Pat. Office, **657,** 5: 1381. 1952.

Structural details are given for a machine for injecting gas under pressure into containers of cream. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. Jenness, section editor

399. Utilizing nutrition research in sales promotion. H. Degraff, Cornell Univ. Milk Dealer, 41, 5: 62–68. Feb., 1952.

A discussion of the nutritive value of milk and milk products shows that these products lend themselves to advertising and sales promotion. The advertising features of these products are taste appeal, high quality protein, vitamin content and, perhaps, the unique Ca content of milk, the strongest of all. Beyond these facts, the products sell at a bargain price as shown by tables comparing costs of steak, eggs and cheese with that of milk. Figures are presented.

C. J. Babcock

400. Effect and distribution of vitamin B_T. G. Fraenkel, Univ. of Ill., Urbana. Arch. Biochem. Biophys., **34,** 2: 457–467. Dec., 1951.

In addition to the known B-vitamins and folic acid, the larvae of the mealworm, *Tenebris molitor,* required for growth and survival a factor designated B_T, found originally in Norite filtrates of yeast and liver extract. Milk and dried whey are superior sources of B_T. Based on solids in the diet, 0.3% milk and 0.15% dried whey exhibit the same order of activity as 1–2% dried brewers' yeast. Casein is devoid of activity. Concentrates of vitamin B_T fail to stimulate rats, chicks, guinea pigs, *Tetrahymena* sp., *Streptococcus faecalis* R, *Leuconostoc citrovorum, Lactobacillus bulgaricus, Bacillus thermoalimentosum* and *Clostridium sporogenes.* It bears some resemblance to the guinea pig factor 3, and its activity differs from that of vitamin B_{12} and folic acid. H. J. Peppler

SANITATION AND CLEANSING

K. G. Weckel, section editor

401. Acid cleaner and detergent. G. E. Brissey and H. H. Young (assignors to Swift & Co.). U. S. Patent 2,593,259. 5 claims. Apr. 15, 1952. Official Gaz. U. S. Pat. Office, **657,** 3: 886. 1952.

Removal of heat-coagulated milk protein films is facilitated by using a cleaner composed of phosphoric acid, an alkali metal acid sulphate and an organic acid between acetic acid and a mineral acid in strength. The mixture is proportioned to obtain a pH of 1.8 or below. R. Whitaker

402. Valveless vacuum-operated fluid circulating device for cleaning hollow objects such as teat cups and milk tubes. F. T. Redman, Jr. (assignor to Hall Labs., Inc.). U. S. Patent 2,595,539. 8 claims. May 6, 1952. Official Gaz. U. S. Pat. Office, **658,** 1: 149. 1952.

Milking machine parts are cleaned by this equipment, which recirculates a cleaning solution through all tubes and cups without dismantling. R. Whitaker

403. Milk sanitation honor roll for 1950–51. Anonymous. Pub. Health Repts., **67,** 3: 268–271. 1952.

A list is presented of 251 cities and counties on the Public Health Service "honor roll" for the period Jan. 1, 1950 to Dec. 31, 1951. A rating of at least 90% compliance with the USPHS Milk Ordinance and Code is necessary for inclusion. The list is made up of 2 parts, those communities in which all market milk is pasteurized and those in which both raw and pasteurized milk are sold. Some communities, although deserving to be included, may not be listed because their ratings haven't been determined by the State milk sanitation authority concerned. D. D. Deane

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