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'l'HE ADEQUACY OF AN ALL-ALFALFA HAY RATION FOR MILK SECRETION" [2]

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Huffman and Duncan (3, 4) reported that milk production can be increased when properly depleted cows have had a part of the alfalfa hay in the ration replaced by concentrates on an equal total digestible nutrient (TDN) basis. These workers postulated that corn grain contains an unidentified factor(s) needed to balance alfalfa hay for milk production.

In 1944, Huffman and Duncan (3) published data from which they concluded that a lack of available energy was not the factor responsible for the low producing power of alfalfa hay. Later, Smith *et al.* (8) reported data from which they concluded that the milk production obtained on alfalfa hay alone was not quite as high as expected when the calculations were made on the net energy basis.

A critical study of these data, however, suggests that the question of the lack of energy as the effective factor has not been excluded definitely. When the data of Smith *et al.* were recalculated according to Kellner's (5) method on the basis of the chemical analyses of the hays, the net energy values obtained for the alfalfa hays were found to be appreciably lower than those used by these authors in their calculations. At the beginning of the trials the cows were underfed considerably and the initial sharp decrease in milk production could be explained on this basis. As milk production approached that expected on the basis of the recalculated net energy intake, milk production leveled off. On the basis of these recalculated values, when concentrates were added to the ration, the variations in milk production appeared to reflect the variations in net energy intake rather closely.

In the earlier report of Huffman and Duncan (3), the analytical data presented on the feeds used are not sufficient for recalculating the net energy values.

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s The experimental data in this paper are taken in part from a thesis submitted by M. A. Sami in partial fulfillment of the requirements for the degree of Master of Science in Dairy Husbandry, University of Maryland.

However, when the net energy values were calculated on the basis of Morrison's average analyses corresponding with the TDN values used by these workers, the amount of net energy added when corn was fed exceeded that received during the all-alfalfa period in all trials. The average increase of net energy in the rations of these nine cows was 3.5 therms per day. This is equivalent to 11.66 lb. of 4 per cent FCM. The actual average increase was only 2.8 lb. These results appear to be about as expected, taking into consideration the fact that several of these cows were overfed before the changes to concentrates were made. In the data presented in 1949 by Huffman and Duncan (4), the analytical data were sufficiently complete to permit direct calculation of the net energy values of the feeds. Fifteen cows were maintained on alfalfa hay alone for 8 wk. or less, after which part of the hay was replaced by an equivalent amount of TDN in the form of corn. An increase in milk production was observed in every case. However, when these data were recalculated to a net energy basis, it was observed that only two of the cows reacted positively and 12 negatively, while one showed no difference. The average milk yield during the corn feeding periods was 3.2 lb. less than expected. This appears to be about the expected normal decline in lactation. When the above data were recalculated on the basis of the Scandinavian system, the results were slightly different but of the same order.

In these same studies, pure corn starch appeared to have less value than corn when calculated on either the TDN or net energy basis. There appear to be a number of possible explanations for these differences. Possibly, the sudden addition of large quantities of soluble carbohydrates to a ration consisting of large amounts of hay decreased the digestibility of the hay, especially of the crude fiber, so that the total net energy effect was less than calculated. In this type of feeding an abnormal rumen fermentation also may be involved, especially if the excessive acids produced in the rumen are not neutralized properly. Digestive disturbances from the feeding of starch and sugar frequently have been reported (6, 1, 2).

Since it still appeared possible that the positive effects of concentrates when added to an alfalfa ration could be due to an increase in net energy intake, an experiment was designed on a net energy basis and precautions were taken to insure that disturbances caused by excessive amounts of soluble carbohydrates or excessive quantities of dry matter in the ration would be avoided.

EXPERIMENTAL

Eight cows in the early stages of lactation, three Ayrshires, two Guernseys and three Holsteins, were used for the experiment. Five of the cows had been on alfalfa hay, corn silage and a 16 per cent grain mixture. The other three, the Ayrshire cows, had been on pasture alone. All of the cows were fed according to Morrison's net energy standards. The higher requirements were used for both maintenance and production. Every effort was made to keep the energy intake at or slightly above the requirements. Four different lots of alfalfa hay were used, of which three were graded U. S. no. 1 and one U. S. no. 2. The analyses of these hays along with other feeds used are presented in table 1. The

TABLE 1

Descrtption of the feeds, their chemical composition and assumed coefficients of digestibility and the calculated TDN and net energy values

	Lot no. 1	Dig. coeff.	Lot no. 2	Dig. coeff.	Lot no. 3	Dig. coeff.	Lot no. 4	Dig. coeff.	Feed cellu-lose	Sucrose	Corn starch	Corn
Description of the feeds	U. S. no. 1		U. S. no. 1		U. S. no. 2		U. S. no. 1		Ruffex			Dent no. 2
	(%)		(%)		(%)		(%)		(%)	(%)	(%)	(%)
Moisture	9.87	.....	10.24	.....	11.12	.....	8.59	.....	6.62	1.0	6.0	15.0
Ash	6.80	.....	6.95	.....	5.63	.....	6.54	.....	0.72	.....	.....	.....
Crude protein	16.45	73	19.66	72	14.35	72	17.81	73	1.07	.....	.....	.....
Ether extract	2.24	33	3.82	39	1.95	31	3.06	32	0.54	.....	.....	.....
Crude fiber	24.54	46	21.71	53	36.11	43	24.41	44	67.75	.....	.....	.....
N. F. E.	40.10	72	37.62	69	30.84	71	39.59	72	23.30	.....	.....	.....
TDN/100 lb.	54.52	.....	53.89	.....	48.39	.....	53.54	.....	74.1	92.1	87.4	80.1
Net energy therms/100 lb.	41.25	.....	43.78	.....	29.18	.....	41.81	.....	61.0	75.0	94.0	80.1

net energy of the various feeds was calculated from these analyses according to Kellner (5), assuming that one starch equivalent is equal to 1,071 Calories of net energy for fattening. The total dry matter intake was limited so that digestion would not be affected adversely. To maintain the dry matter intake at the desired level, a purified carbohydrate mixture was fed in addition to the hay. The addition of the purified carbohydrate mixture had the added advantage of lessening the amount of any unknown factor(s) which would be received from the alfalfa hay. Two of the cows were low producers and received alfalfa hay alone for 8 wk., because the amount of hay needed to supply the required net energy was relatively low and therefore the intake of dry matter was not considered excessive.

The carbohydrate mixture consisted of 70 per cent commercial rice straw cellulose (Ruffex), 18 per cent cane sugar and 12 per cent corn starch. The bulk of the carbohydrate mixture was made up of cellulose in order to avoid digestive disturbances and the depression of the digestibility of crude fiber. Cane sugar was used instead of corn sugar because it increased the palatability of the mixture. When hay or concentrates were replaced by the purified carbohydrate mixture, the change-over was made gradually over a period of 1 wk. or 10 days. When the carbohydrate mixture was replaced by corn, the change was again made gradually over a period of 1 wk. For some of the cows a small amount of cottonseed oil was added to the carbohydrate mixture. A sufficient amount of hay always was fed to maintain an adequate protein intake. Each cow received daily 300 g. of bone meal and 50 to 60 g. of salt mixed with the concentrates or mixed with hay when hay alone was fed.

The cows were fed and milked twice daily. Milking was done by machine. In the beginning, 1-day milk samples were taken at 3-day intervals. Later composite samples were taken for 3 consecutive days each week. Feed changes were made once a week, based upon the average daily production of the previous week. During the control and experimental periods, the net energy intake was kept as constant as possible. The cows were weighed once every week at the same hour on the morning of the day that feed adjustments were made.

The net energy value of the sugar and starch was evaluated in accordance with Huffman and Duncan (3) after determining the moisture content. The digestibility of fat was assumed to be 90 per cent, and its energy value 4.6 calories per milliliter. Since the net energy value of corn is relatively constant, the value for net energy given by Morrison was used and only the dry matter was checked. For cellulose the average net energy value was used and it was calculated from the Scandinavian feed unit value reported by Larson (7), based on feeding trials with cattle. One digestion trial (unpublished) indicated that the value may be too high for Ruffex. If this proves to be true in later trials, the results obtained by the replacement of this mixture with corn will be even less favorable for corn. The period test method was used in calculating the results. The last week before the change-over and the first week after the change-over were used as control periods. The first week during each corn period was considered as a change-over period and was not used in the calculations.

RESULTS

All of the cows were maintained on Morrison's higher net energy standards throughout the experiment. This was reflected in very uniform body weights. The average live weights of the eight cows, based upon the first two and last two weighings were 1,069 and 1,074 lb., respectively.

No abnormal decrease was observed in the lactation curves of the two cows which were maintained on alfalfa hay alone for 8 wk. Likewise, the lactation curves were normal for the six cows receiving alfalfa hay plus a purified carbohydrate mixture or the carbohydrate mixture and fat for from 56 to 154 days. The cows were in various stages of lactation ranging from 33 to 117 days when placed on experiment.

The results of the changes from the hay and purified carbohydrate mixture to hay and corn are shown in tables 2 and 3. The essential production and feeding data are shown in table 2, whereas, a more concise summary of the results is shown in table 3. The production and feeding data in table 2 are given separately for both control and experimental periods. The control period in each case represents an average of the week preceding the corn period, and the first week after changing back to the control ration and the values were interpolated so that they correspond to the time of the corn feeding period. The corn periods (experimental) varied from 3 to 5 wk., in which the first week was used as the change-over period and the data excluded from the calculations. Due to the fact that the net energy was kept constant during the control and test periods, the cows were slightly over-fed during these periods.

The increases or decreases in the net energy content of the rations during the corn feeding period were calculated and expressed as the expected changes in milk yield, assuming that 1 lb. of 4 per cent F.C.M. is equivalent to 0.3 therms of net energy for fattening. These values are compared in table 3, with the actual changes in milk yield. It will be observed that these values are closely related. The average expected change was – 1.11 lb. and the average actual change – 0.50 lb. Milk production changes paralleled the net energy changes so closely that the variations in the net energy intake explain the results obtained quite adequately. It is possible, of course, that the hays used in this investigation were not deficient in the factor(s) reported by previous workers.

It could be postulated that the purified carbohydrate mixture used in all cases or the cottonseed oil used in six cases contained an essential factor not present in alfalfa hay. That such was not the case is shown by the fact that when the two cows on alfalfa hay alone were changed on the net energy basis to hay and fat and then to hay and a carbohydrate mixture (table 4) there was no indication that the rate of decrease in milk yield was retarded.

SUMMARY AND CONCLUSIONS

When eight cows were fed according to their net energy requirements from 56 to 154 days on either alfalfa hay alone or in combination with a purified carbohydrate or carbohydrate and fat mixture, so that the amount of dry matter in the ration was not excessive, no abnormal decrease in milk yield was observed.

TABLE 2

The data pertaining to body weights, average daily yield of 4% FCM., and intakes of alfalfa hay, carbohydrate mixture, fat and corn and the TDN and net energy received and required

Trial no.	Cow	Days on experiment[a]	Control or corn period[b]	Length of test period	Body wt.	FCM	Hay	CHO mixture	C. oil	Corn	TDN		Net energy	
											Rec.	Req.	Rec.	Req.
				(*wk.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*ml.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*therms*)	
1	Judy	105	Control		1164	6.3	22.5	4.0	.....	.....	15.2	11.0	12.3	9.2
			Corn	4	1158	5.2	23.0	.....	.....	3.20	14.9	10.7	12.2	8.9
2	Tydings	105	Control		951	11.7	20.0	6.0	.....	.....	15.4	11.3	12.7	9.6
			Corn	4	945	10.6	19.0	.....	.....	5.20	14.3	11.0	12.1	9.3
3	Faith	119	Control		1160	19.1	24.5	5.2	290	.....	17.7	15.1	15.1	13.0
			Corn	2	1164	16.3	24.0	.....	.....	5.46	17.2	14.2	14.4	12.1
4	Faith	154	Control		1197	16.7	24.0	6.0	140	.....	17.8	14.2	14.6	12.4
			Corn	2	1199	17.3	24.0	.....	.....	5.46	17.2	14.9	14.4	12.6
5	Cherry	119	Control		896	13.9	22.0	2.7	145	.....	14.2	12.2	11.7	10.4
			Corn	2	991	13.3	21.0	.....	.....	3.37	13.9	12.0	11.5	10.2
6	Cherry	154	Control		987	10.1	21.0	3.0	70	.....	13.7	11.0	11.1	9.2
			Corn	2	991	11.0	21.0	.....	.....	3.37	13.4	11.3	11.5	9.6
7	Pomona	56	Control		870	22.5	22.0	8.0	.....	.....	18.1	14.2	14.6	12.4
			Corn	3	858	24.0	22.0	.....	.....	6.80	17.2	14.7	14.6	12.8
8	Lizzie	56	Control		877	17.3	16.0	8.0	.....	.....	14.9	12.6	12.1	10.8
			Corn	3	875	16.5	16.0	.....	.....	6.80	14.0	12.3	12.1	10.6
9	Roberta	63	Control		1248	16.9	24.0	6.0	140	.....	17.8	15.0	14.7	12.8
			Corn	2	1248	16.9	24.0	.....	.....	5.20	17.0	15.0	14.2	12.8
10	Romona	63	Control		1269	34.5	28.0	10.0	280	.....	23.4	20.7	18.1	18.1
			Corn	2	1250	32.9	28.0	.....	.....	8.50	21.8	20.2	16.6	17.6

[a] Before change-over to corn.

[b] The last week before the change-over to corn and the first week after changing back to the control ration were used as the control periods and the average values of these two periods were interpolated so that they correspond to the time of the corn feeding period.

TABLE 3

The differences in the amounts of net energy in the rations during control and test periods, the expected and actual changes in the milk yields and the changes in body weights

Trial	Cow	Alfalfa hay		Carbohydrate mixture removed	Corn added	Expected change in FCM	Actual change in FCM	Change in body wt.
		Control period	Corn period					
		(*therms*)	(*therms*)	(*therms*)	(*therms*)	(*lb.*)	(*lb.*)	(*lb.*)
1	Judy	9.62	9.62	− 2.72	+ 2.56	− 0.5	− 1.1	− 6
2	Tydings	8.57	7.95	− 4.08	+ 4.16	− 1.8	− 1.1	− 6
3	Faith	10.23	10.00	− 4.82	+ 4.37	− 2.3	− 2.8	+ 4
4	Faith	10.00	10.00	− 4.62	+ 4.37	− 0.8	+ 0.6	+ 2
5	Cherry	9.20	8.78	− 2.50	+ 2.70	− 0.7	− 0.6	+ 5
6	Cherry	8.78	8.78	− 2.36	+ 2.70	+ 1.1	+ 0.9	+ 4
7	Pomona	9.20	9.20	− 5.44	+ 5.44	0.0	+ 1.5	− 12
8	Lizzie	6.69	6.69	− 5.44	+ 5.44	0.0	− 0.8	− 2
9	Roberta	10.00	10.00	− 4.72	+ 4.17	− 1.8	0.0	0
10	Romona	10.00	10.00	− 8.09	+ 6.81	− 4.3	− 1.6	− 19
	Av.	9.23	9.10	− 4.48	+ 4.27	− 1.11	− 0.50	− 2.8

TABLE 4

The effect on milk yield of the replacement of hay with oil and with a purified carbohydrate mixture

Cow	Exptl. period	Feeding	Body wt.	FCM.	Net energy	
					Rec.	Req.
	(*wk.*)		(*lb.*)	(*lb.*)	(*therms*)	(*therms*)
Judy	3	35.5 lb. alfalfa hay	1,160	16.1	14.2	12.0
	3	22.0 lb. hay + 225 ml. oil	1,154	14.9	14.2	11.7
	3	16.3 lb. hay + 6.4 lb. carbohydrate mixture	1,138	11.8	14.2	10.7
Tydings	3	34.0 lb. alfalfa hay	952	26.1	13.3	13.9
	3	30.0 lb. hay + 225 ml. oil	961	22.0	13.1	12.7
	3	21.0 lb. hay + 6.4 lb. carbohydrate mixture	954	16.3	13.4	11.0

In ten trials the replacement of the purified mixtures with corn did not result in any changes in milk production which could not be explained on the basis of the calculated net energy intake.

In two trials there was no increase in milk production when alfalfa hay was replaced by either fat or a purified carbohydrate mixture.

Calculations indicate that the milk production-increasing effect of corn and other concentrates added to an all-alfalfa ration as reported in previous papers can be explained on a net energy basis.

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VITAMIN B_{12} IN BLOOD OF MATURE DAIRY COWS LIBERALLY FED GRAIN, ALFALFA HAY, SILAGE AND PASTURE FORAGE AS MEASURED BY LACTOBACILLUS LEICHMANNII 4797 (ATCC)[1, 2]

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Numerous reports have appeared concerning the nutritive properties of vitamin B_{12} for various species since its isolation (11, 15). Study of the blood level of a particular constituent has been widely used as a research tool by the animal nutritionist. For example, the vitamin content of blood from various species has been determined and has been shown to be related to the content of such vitamins in the diet. Moore (6) reported on the level of blood plasma carotene in dairy calves as related to carotene intake. The requirement of vitamin A for normal growth in young dairy calves was investigated by Converse and Meigs (1). The changes in blood plasma vitamin A of dairy cattle on winter rations was reported by Phillips *et al.* (9). Data on normal blood level of niacin for Guernsey, Jersey and Holstein calves were reported by Teeri *et al.* (17). Pearson *et al.* (7) studied the metabolism of niacin in sheep and reported data on blood level of the vitamin for this species. The normal pantothenic acid content of the blood of the dog, horse, human, pig, rabbit and sheep were reported by Pearson (8). Information concerning riboflavin in blood was reported by Ray *et al.* (10) and Strong *et al.* (16). Eaton *et al.* (3, 4) investigated the influence of the diet of dairy cattle on blood levels of vitamin D. Schweigert and Pearson (12) determined the folic acid content of blood from various species and found cattle to have a much lower blood level of this vitamin than did the pig, chicken and turkey.

Couch *et al.* (2) determined the vitamin B_{12} content of the blood from various species. Blood from cattle on pasture was found to contain, on the average, 0.5 mμg. of vitamin B_{12} activity per milliliter, while blood from calves receiving a fattening ration in dry lot contained 0.9 mμg. of vitamin B_{12} activity per milliliter.

Because of the apparent variation in blood vitamin B_{12} of cattle fed different rations (2), and the need for more extensive information on the role of vitamin B_{12} for dairy cattle, the present study was undertaken. The work was planned to furnish basic information on the normal blood level of vitamin B_{12} for mature dairy cattle liberally fed well-balanced rations. Although it is recognized that

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[1] Supported in part by Project RG 1862, Division of Research Grants and Fellowships, U. S. Public Health Service, National Institute of Health, Bethesda, Md.

[2] The crystalline vitamin B_{12} used in the microbiological assays was generously supplied by D. F. Green, Veterinary Dept., Merck and Co., Rahway, N. J.

[3] Department of Dairy Husbandry.

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Brahman cattle should not be confused with the dairy breeds, their inclusion in this study results from the present widespread interest in crossing this breed with the European dairy breeds.

EXPERIMENTAL PROCEDURE

Results of analyses on blood of purebred Holstein, Jersey, Guernsey and Brahman breeds are reported. The Holstein and Jersey cows were in the A. & M. College milking herd. The ration of these cows consisted of liberal feeding of grain, good quality alfalfa hay, sorghum silage and some sudan grass pasture. The grain was home-mixed and contained cottonseed meal, ground oats, ground milo, wheat bran, bonemeal, salt and a trace mineral mixture. The Brahman cows were not in milk when the samples were collected. These cattle were owned by the College or were on loan for use in a Regional Crossbreeding Project and are mostly purebreds, but include both Gir and Guzerat breeds. The Brahman cattle were being fed, at the time of sampling, a grain mixture similar to that fed the milking herd. In addition, the animals were grazing good Bermuda, Dallis and bluestem grass pasture. The pasture was not lush but could be rated good. The Brahman cattle ranged in age from 1 to 4 yr. The Guernsey cattle employed in this experiment were owned by a local dairy company. The cows were in milk and were fed a commercial dairy mix (containing 15 per cent protein), alfalfa hay and legume-grass pasture. All the Guernsey cows were registered and had been bred by the time the blood samples were collected.

Blood samples were collected in oxalated tubes from the jugular vein of test animals immediately following the afternoon milking. The samples were refrigerated overnight and the analyses were begun the following morning. Procedure for preparation of stock solutions for microbiological assay for B_{12} was the same as previously reported by Couch *et al.* (2), except that 3 ml. of blood were used in preparing the stock solutions. The assay for vitamin B_{12} activity was conducted according to the procedure of Skeggs *et al.* (13).

Three ml. of oxalated whole blood were pipetted into 50 ml. of water in a 125-ml. flask. Ten ml. of a pancreatin solution (3 mg. per milliliter) were added and the solution was covered with toluene. The flask was stoppered with cork and placed in a shaker at 37.5° C. for approximately 24 hr. The samples then were autoclaved for 5 min. at 15 lb. pressure. After cooling, the samples were made up to 100 ml. and filtered. The filtrates were covered with toluene and stored at approximately 20° C. The filtrates constituted the stock solutions from which dilutions were made for the microbiological assays for vitamin B_{12}. In other studies it was determined that the above mentioned procedure gave identical results with those obtained when a pH of 7.2 to 7.4 was used during the digestion with pancreatin. *Lactobacillus leichmannii* 4797 (ATCC) was the test organism, and acid production was the criterion used to measure growth response. Crystalline vitamin B_{12} was used as the standard in all of the assays mentioned in this report.

RESULTS AND DISCUSSION

From the results of this study (table 1), it is apparent that the vitamin B_{12} content of Jersey blood is lower than that of the Holstein, Guernsey and Brah-

man cattle. There was no difference in the B_{12} content of the blood of pregnant and open females in either the Jersey or Holstein breeds. Couch *et al.* (2) reported that the B_{12} content of blood from cattle on pasture during the dry season was only 0.5 mμg. per milliliter blood, while that from beef calves that were dry lot fed contained 0.9 mμg. of the vitamin per milliliter of blood.

The Holsteins and Jerseys in the present report were maintained under practically identical dietary regimes in the college herd, yet the B_{12} content of the blood from the Holstein is significantly higher than that of the Jersey. No explanation is immediately available as to why this difference in the B_{12} content of the blood from these two breeds should exist. It is fully realized that the Holstein is a larger animal but this does not seem to be a plausible explanation for the higher B_{12} content in the blood of this breed over that of the Jersey, as noted in the present study.

TABLE 1

The vitamin B_{12} content of blood from mature cows of different breeds fed liberally a grain mixture, alfalfa hay and sorghum silage and allowed to graze on sudan grass pasture

Breed	No. cows	Range	Average
	Vitamin B_{12} (*mμg./ml. blood*)		
Jersey (pregnant)	9	0.44 – 1.10	0.73 ± 0.06
Jersey (open)	10	0.45 – 1.10	0.71 ± 0.05
All Jerseys	19	0.44 – 1.10	0.72 ± 0.04
Holstein (pregnant)	10	0.52 – 1.40	1.03 ± 0.09
Holstein (open)	9	0.60 – 1.50	1.04 ± 0.09
All Holsteins	19	0.52 – 1.50	1.03 ± 0.06
Guernsey	10	0.70 – 1.40	1.05 ± 0.07
Brahman	23	0.80 – 1.60	1.19 ± 0.05

The work of Skeggs *et al.* (13), Snell *et al.* (14) and Hoffman *et al.* (5) has shown that *Lactobacillus leichmannii* responds to thymidine. Hoffman *et al.* (5) has shown that vitamin B_{12} is destroyed by autoclaving in an alkaline medium. Couch *et al.* (2) reported that the B_{12} content of calf blood is completely destroyed by autoclaving at pH 12.0 and, therefore, it is believed that the B_{12} content (as measured with *Lactobacillus leichmannii*) of cattle blood as determined in the present report is due to B_{12} in the blood and not to thymine desoxyriboside. Couch *et al.* (2) showed further that from 70 to 83 per cent of the *L. leichmannii* activity of blood from species with nucleated erythrocytes (chicken, turkey and turtle) remained after alkaline autoclaving. This fraction of the activity is thought to have been due to thymidine in the nucleated erythrocytes.

SUMMARY

The vitamin B_{12} content of blood from Jersey, Guernsey, Holstein and Brahman cattle was determined by microbiological assay, and the average values found were 0.72, 1.05, 1.03 and 1.19 millimicrograms B_{12} per milliliter of blood for the four breeds, respectively.

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EFFECTS OF METHOD OF PRESERVATION OF ROUGHAGE, AND OF CANE OR WOOD MOLASSES ON VITAMIN EXCRETION BY COWS[1]

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It is known that diet influences the intestinal flora of certain species of animals (2) and that certain vitamins of the B complex are synthesized by microorganisms in the digestive tract (3). It generally is assumed that such synthetic activity makes ruminants independent of a dietary source of many of the water-soluble vitamins (5). However, this independence could be seriously affected by any factor which might alter the rumen or intestinal flora. The influence of ration upon the establishment of certain microorganisms in the rumen of young calves recently has been reported (6, 7), and it was suggested that early development of rumen function has a favorable effect in meeting the vitamin needs of young calves (4). It appears, therefore, that knowledge concerning the influence of ration on rumen or intestinal synthesis of vitamins would be of value.

A recent study of the effect of sulfathalidine on the excretion of vitamins by ruminants (8) indicated that fecal and urinary excretion values can be used, in a comparative sense, as a measure of vitamin synthesis, when the animals are maintained on a constant daily intake. In the present investigation this technique has been employed to study the effect of method of preservation and storage of hay in the synthesis of nicotinic acid, thiamine, panthothenic acid and riboflavin in the rumen of cows fed the hay. The effects of including cane and wood molasses in the ration also were studied.

EXPERIMENTAL

General. Three Guernsey heifers (no. 1, 2 and 3), one Holstein (no. 4) and one Ayrshire (no. 5) were used in this study. All were approximately 2 yr. of age. By maintaining the animals in metabolism stalls it was possible to make regular collections of 24-hr. urine and feces samples. Daily aliquots were preserved (by freezing for the feces and by refrigeration at a temperature just above freezing for urine) until five consecutive 24-hr. samples had been obtained. The five aliquots then were pooled, and each experiment involved analysis of three such pooled samples. This made a total of 15 consecutive days upon which calculations of average 24-hr. excretion values were based. A preliminary feeding period of at least 1 wk. served to adjust the animals to their rations which, except when supplemented with molasses, consisted solely of roughage, water and salt. The roughage all came from the same cutting of a stand of mixed timothy hay, ladino, alsike and red clovers and differed only in that one portion was field-cured, another was mow-cured and the third was ensiled by the wilting method. When the effect of molasses was being studied, only the field-cured hay

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was fed. Analysis of these materials indicated average daily vitamin intakes as shown in table 1.

TABLE 1

Average vitamin intakes
(mg./day)

Ration fed	Nicotintc acid	Pantothenic acid	Thiamine	Riboflavin
Field-cured hay	270.0	57.0	4.4	59.0
Mow-cured hay	240.0	60.0	5.0	75.0
Silage	188.0	66.0	3.8	76.0
Cane molasses added	314.0	88.0	5.3	.....
Wood molasses added	336.0	59.0	4.5	.....

Analytical methods. All vitamin analyses were made by the microbiological procedures cited in a previous study (8).

RESULTS AND DISCUSSION

Table 2 presents the average vitamin excretion values obtained when the rations consisted of roughage preserved by field curing, mow curing or ensiling. The excretion of nicotinic acid was relatively constant on all three rations, the differences which did occur not being significant. The excretion of this vitamin did not reflect the decreased intake with silage. However, this fact, has little significance in regard to rumen synthesis, since the excretion, in all cases, was considerably less than the daily intake. As previously suggested (8), it appears that intestinal or rumen synthesis of nicotinic acid generally is not of great importance to the bovine species.

The thiamine excretion values were not significantly altered by feeding the different rations. The slight increase noted when silage was fed attains greater significance, however, when the lessened daily intake with that ration is considered. This could indicate an increased synthetic activity with respect to thiamine by microorganisms in the digestive tract when the ration consists of silage. The fact that in all cases the excretion of thiamine exceeded the daily intake suggests the probable importance of rumen synthesis of this vitamin.

Whether the roughage was field-cured, mow-cured or ensiled had no statistically significant effect upon the excretion of either pantothenic acid or riboflavin. In the case of pantothenic acid, however, the total excretion always exceeded the daily intake. This fact, as has been suggested previously (8), indicates that synthesis of this vitamin in the digestive tract may be of importance in ruminant nutrition.

Table 3 presents the average excretion values obtained when cane and wood molasses were incorporated in the diet. There is no indication that rumen synthesis of either nicotinic acid or thiamine was affected by the presence of these substances. The increased excretions which occurred were completely accounted for by the increased vitamin intake due to the molasses.

The excretion of pantothenic acid was increased significantly when molasses was fed. This increase greatly exceeded the added intake due to the vita-

TABLE 2

Vitamin excretions of animals on the different roughages

Cow no.	Feces			Urine		
	Field-cured	Mow-cured	Silage	Field-cured	Mow-cured	Silage
			Nicotinic acid (*mg./day*)			
1	33.0	.....	38.4	18.7	.....	14.2
2	34.2	34.1	36.2	16.6	21.6	20.3
3	.....	33.0	34.0	.....	30.7	20.6
4	31.1	34.5	.....	23.8	22.4	.....
Av.	32.6	33.9	36.2	19.7	24.9	18.4
			Thiamine (*mg./day*)			
1	3.0	.....	5.4	5.5	.....	6.5
2	4.1	4.1	5.0	3.6	5.7	5.0
3	.....	4.1	3.2	.....	4.1	5.4
4	5.4	5.3	.....	4.6	4.0	.....
Av.	4.2	4.5	4.5	4.6	4.6	5.6
			Pantothenic acid (*mg./day*)			
1	10.8	.....	13.9	82.0	.....	76.5
2	17.4	16.1	20.4	75.2	80.6	84.9
3	.....	14.9	11.6	.....	110.4	70.1
4	21.4	19.5	.....	66.8	56.5	.....
Av.	16.5	16.8	15.3	74.7	82.5	77.2
			Riboflavin (*mg./day*)			
1	12.9	.....	15.0	11.7	.....	12.9
2	11.7	13.3	15.9	9.5	11.3	13.8
3	.....	14.1	16.3	.....	11.2	13.4
4	13.4	12.4	.....	8.9	13.5	.....
Av.	12.7	13.3	15.7	10.0	12.0	13.4

TABLE 3

Vitamin excretions of cows on molasses supplements

Cow no.	Feces			Urine		
	Controls	Cane molasses	Wood molasses	Controls	Cane molasses	Wood molasses
			Nicotinic acid (*mg./day*)			
2	34.2	.....	41.2	16.6	.....	15.7
3	33.0	50.0	42.7	30.7	39.7	23.7
4	31.1	47.5	42.3	23.8	40.8	30.9
5	.....	53.2	50.4	.....	46.3	34.2
Av.	32.8	50.2	44.2	23.7	42.3	26.1
			Thiamine (*mg./day*)			
2	4.1	.....	5.2	3.6	.....	3.7
3	4.1	3.6	4.3	4.1	5.4	4.6
4	5.4	4.9	4.1	4.6	5.4	6.3
5	.....	5.0	4.0	.....	5.3	6.4
Av.	4.5	4.5	4.4	4.1	5.4	5.2
			Pantothenic acid (*mg./day*)			
2	17.4	.....	34.6	75.2	.....	70.4
3	14.9	22.5	24.6	110.4	110.1	69.7
4	21.4	33.4	27.1	66.8	110.0	100.8
5	.....	52.9	54.4	.....	134.3	100.8
Av.	17.9	36.3	35.2	84.1	118.1	85.4

min content of the molasses and, therefore, must have resulted from increased synthetic activity in the digestive tract. Since the same result was obtained with both cane and wood molasses, it is suggested that the microorganisms which synthesize pantothenic acid are favorably affected by a high carbohydrate medium.

Based upon protein and energy digestion balance experiments, it has been reported (1) that wood molasses is comparable to cane molasses as a feed for dairy cattle. The present study offers the additional information that wood molasses is comparable to cane molasses with respect to its effect upon the rumen or intestinal synthesis of nicotinic acid, thiamine and pantothenic acid.

SUMMARY

Three methods of preservation and storage of hay were compared with respect to their effect upon vitamin synthesis in the digestive tract of cows fed the hays. Fecal and urinary excretions of the vitamins were measured. Whether the roughage was field-cured, mow-cured or ensiled had no significant effect upon the excretion of nicotinic acid, pantothenic acid or riboflavin. Silage apparently favorably affected the synthesis of thiamine in the digestive tract.

As a feed for dairy cattle, wood molasses was found to be comparable to cane molasses with respect to its effect upon rumen or intestinal synthesis of nicotinic acid, thiamine and pantothenic acid. In the case of pantothenic acid, this synthetic activity was considerably increased when molasses was present.

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CONGO RED AS AN INHIBITOR FOR QUATERNARY AMMONIUM COMPOUNDS[1]

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Although many investigators have studied quaternary ammonium compounds during the last decade, agreement has not been reached on a method which will indicate accurately the germicidal activity of quaternaries. Many factors, as reviewed by Rahn and Van Eseltine (2), influence the action of quaternaries on bacteria and must be considered under various experimental and practical conditions to minimize variations in results. The bacteriostatic action of quaternary ammonium compounds greatly increases the need for using inhibitors for inactivating these compounds when assessing their germicidal activity. Weber and Black (3) compared several inhibitors and found lecithin to be the most satisfactory. However, lecithin was not found by Weber and Black (5) to protect all types of bacteria equally, and due to difficulties in dispersion the usable concentrations of lecithin were limited. The difficulties encountered in dispersing lecithin, even with the use of Tween 80 (polyoxyethylene sorbitan monooleate), and variations in the chemical composition of commercial preparations of lecithin make it difficult to prepare known concentrations of this compound. The use of a synthetic and chemically defined inhibitor which is water-soluble at the concentrations to be employed would be advantageous for studying the germicidal values of quaternary ammonium compounds. Consequently, congo red, a certified, acidic anionic dye which has not been reported previously as an inhibitor, has been used in this laboratory. A comparison of congo red to lecithin showed that congo red has possibilities for application as an inhibitor for quaternaries.

EXPERIMENTAL

Escherichia coli 198[4] and *Micrococcus pyogenes* var. *aureus* 209[4] were used as test organisms with four quaternaries of different chemical compositions. The latter will be referred to as quaternary A (alkenyl dimethyl benzyl ammonium chloride), quaternary B (alkyl dimethyl benzyl ammonium chloride), quaternary C (N (acyl colamino formyl methyl) pyridinium chloride) and quaternary D (methyl dodecyl benzyl trimethyl ammonium chloride). Ten per cent commercial preparations of the quaternaries were used. The various concentrations used in this study were obtained by dilution and were not substantiated by analysis.

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To determine the effect of congo red on the test organisms, plate counts were made on tryptone-glucose-beef extract agar after exposing *E. coli* and *M. pyogenes* var. *aureus* for 2 hr. at 25° C. to sterile aqueous solutions containing as great as 400 ppm. of congo red. In some instances, the numbers of test bacteria after exposure to 400 ppm. of congo red for 2 hr. were about 20 per cent lower than in cases in which congo red was not used. However, the reduction in numbers of bacteria was not constant, and it was concluded that congo red did not have sufficient germicidal action on the test bacteria to reduce the usefulness of this dye as a neutralizer of quaternaries.

Preliminary experiments in which congo red was used as an inhibitor for quaternary A were carried out using visual observation to determine the growth of bacteria which survived treatment with quaternary. Quantities of 5 ml. of 18-hr. broth culture of *E. coli* were adjusted to 30° C., inoculated into test tubes containing 5 ml. of 50 and 100 ppm. of quaternary A and held in a water bath at 30° C. for periods up to 60 min. After each exposure, 0.1 ml. of a mixture was subcultured in 9.9 ml. of tryptone-glucose-beef extract broth. Growth of *E. coli* in the subculture medium at 48 hr. was irregular after treatment with 50 ppm. of quaternary and all results were negative for two trials with 100 ppm. of quaternary. However, when 150 ppm. of congo red were included in the subculture tubes, growth occurred in all cases, even after an exposure of 60 min. to 100 ppm. of quaternary A.

Since the determination of survival of organisms by visual observation of growth in tubes of broth revealed no information as to the numbers of surviving bacteria, the method of Weber and Black (4), with slight variations, was used for subsequent work. This method bases the evaluation of quaternaries upon the numbers of test bacteria which survive exposure to quaternaries. In this study inhibitors were present only in the dilution blanks, and glucose was omitted from the tryptone-beef extract agar used for determining plate counts. In all cases, sterile distilled water was used to avoid interference by metallic ions. All solutions of quaternaries, and of inhibitors, and also the bacterial suspensions were adjusted to 25° C. in a water bath. Five ml. of a solution containing 100 ppm. of quaternary were added to and mixed with 5 ml. of a 1:10 dilution of a 24-hr. tryptone-beef extract broth culture of test bacteria. Thus, the standard concentration of quaternary permitted to contact the bacteria was 50 ppm. After exposing bacteria to quaternary, dilutions of 1:10 were made in distilled water containing inhibitor, and the concentration of quaternary contacted by the inhibitor was 5 ppm. A standard reaction time of 2 min. was allowed for neutralization of quaternary prior to continuation of the plating procedure. Two min. were found sufficient for neutralization of quaternary, reaction periods of 15 and 30 min. not resulting in higher plate counts than when the reaction period was only 2 min. Immediately prior to each experiment the number of test bacteria in the diluted inoculum was determined by the plate count. The value obtained was divided by two to indicate the number of bacteria present in the mixture of quaternary and bacteria prior to the action of quaternary. This result was considered a control value and was used for comparison of the numbers of bacteria

which survived treatment with quaternary. This method for determinig control values was used because it facilitated the running of experiments and because it previously had been determined that congo red was not significantly toxic to the test bacteria under more drastic conditions than those used in these experiments.

Since Weber and Black (3) had found lecithin to be the most effective inhibitor of quaternaries used in their study, a comparison was made between 5, 50 and 200 ppm. of congo red and 500 ppm. of lecithin (asolectin) using *M. pyogenes* var. *aureus* 209 as test strain. Tween 20 (polyoxyethylene sorbitan monolaurate) was used as a dispersant for lecithin. Solutions of inhibitors were dispensed in quantities of 9 ml., sterilized by autoclaving and employed as dilution blanks. The ratios of quaternary to inhibitor were 1 : 1, 1 : 10 and 1 : 40 for congo red and 1 : 100, as recommended by Weber and Black, for lecithin. Since all trials were not performed on the same day and there were differences between the control plate counts, the results of each trial were multiplied by the factor necessary to translate the numbers of bacteria of each trial in terms of a control culture containing 1×10^8 bacteria per milliliter. The values obtained by such calculations were easily compared and were only slightly different from the actual experimental results. Data from three trials using 5, three trials using 50 and five trials using 200 ppm. of congo red, and six trials using 500 ppm. of lecithin were averaged logarithmically and the results are given in table 1. The numbers of

TABLE 1

Numbers of Micrococcus pyogenes var. aureus per ml. using congo red and lecithin as neutralizers for 5 ppm. of quaternary B

Exposure (*sec.*)	Plate counts using:			
	Congo red			Lecithin
	5 ppm.	50 ppm.	200 ppm.	500 ppm.
Control (0)	1.0×10^8	1.0×10^8	1.0×10^8	1.0×10^8
15	3.0×10^4	7.0×10^5	5.3×10^5	1.9×10^5
30	9.0×10^3	5.5×10^4	7.0×10^4	2.7×10^4
60	7.2×10^2	1.5×10^4	5.0×10^3	1.9×10^3
120	5.9×10^0	4.2×10^2	2.3×10^2	1.9×10^2
300	0.0×10^0	3.0×10^0	1.7×10^0	2.4×10^0

bacteria which survived exposure to 50 ppm. of quaternary B were slightly greater at 15, 30 and 60 sec. with 50 and 200 ppm. of congo red than with 500 ppm. of lecithin, and use of 500 ppm. of lecithin as the inhibitor resulted in greater numbers of viable bacteria throughout the entire 300 sec. than did the use of 5 ppm. of congo red. Thus, a concentration of inhibitor in the 9-ml. dilution blanks which resulted in a ratio of congo red to quaternary of 1 : 1 seemed less adequate for immediate neutralization of the quaternary than either a ratio of congo red to quaternary of 10 : 1 or 40 : 1 or a ratio of lecithin to quaternary of 100 : 1. In all cases, the numbers of bacteria which formed colonies after treatment with 50 ppm. of quaternary B decreased rapidly as the period of exposure was increased. This seemed to indicate that the action of the inhibitors was only to neutralize residual quaternary and thereby protect the bacteria not already inactivated.

If the action of congo red were only the protection of bacteria by neutralizing residual quaternary, it seemed that if sufficient amounts of congo red were mixed with quaternary prior to adding the test organisms, the quaternary would be neutralized, and the mixture should have little or no effect on bacteria added subsequently. Precipitation occurred in cases of mixing 100 ppm. of congo red and 125 ppm. of quaternary B, and the solution became quite clear. With the addition of 25 and 50 ppm. quaternary B to 100 ppm. congo red, no visible precipitation occurred and the mixture retained the red color characteristic of congo red. To determine the effect of mixtures of quaternary B and congo red on *M. pyogenes* var. *aureus* and *E. coli*, concentrations of 0, 100 and 200 ppm. of congo red were mixed with concentrations of quaternary B as high as 200 ppm. After the test organisms had been allowed to remain in contact with the mixtures for 30 sec., plate counts were made. Results of one of several trials with *M. pyogenes* var. *aureus* are given in table 2; results with *E. coli* were similar to those reported for *M. pyogenes* var. *aureus*.

TABLE 2

Effect on numbers of Micrococcus pyogenes var. aureus of exposure to mixtures of quaternary B and congo red for 30 sec.

Quaternary B (*ppm.*)	Plate counts after using Congo red concentrations of:		
	0 ppm.	100 ppm.	200 ppm.
0	1.5×10^{9}	.....	.....
25	4.0×10^{4}	3.2×10^{8a}	2.4×10^{8a}
50	2.0×10^{4}	3.2×10^{8a}	5.6×10^{8a}
100	0×10^{1}	3.1×10^{8a}	2.6×10^{8a}
125	0×10^{1}	9.2×10^{3}	5.1×10^{8a}
150	0×10^{1}	1.1×10^{3}	1.6×10^{8a}
175	0×10^{1}	0×10^{1}	1.1×10^{8a}
200	0×10^{1}	0×10^{1}	4.3×10^{7a}

[a] Estimated count. The number of colonies per plate was much greater than 300.

There was a marked reduction in numbers of bacteria in the case of 25 ppm. of quarternary B and 0 ppm. of congo red, and with 100 ppm. of congo red the decrease in numbers of bacteria was quite significant at 125 ppm. of quaternary B. A concentration of 25 ppm. of quaternary B alone caused approximately as great a reduction in the number of bacteria as did the mixture of 125 ppm. of quaternary B and 100 ppm. of congo red. The data support the conclusion that congo red prevents the inactivation of bacteria by neutralizing quaternary B. In an additional experiment, neither a mixture of 25 ppm. of quaternary B and 100 ppm. of congo red, nor a mixture of 25 ppm. of quaternary B and 250 ppm. of congo red caused decreases in the number of *E. coli* or *M. pyogenes* var. *aureus* during an exposure period of 2 hr. This further indicated the action of congo red in preventing the destruction of bacteria by neutralizing quaternary B.

Since there are numerous reports concerning the variations in germicidal efficiency of different quaternaries and since there are conflicting data as to the resistance of different types of bacteria to quaternaries, the effects of chemically different quaternaries B and C were determined, using *E. coli* and *M. pyogenes*

var. *aureus* as test bacteria and 200 ppm. of congo red as the inhibitor. The reductions in numbers of *M. pyogenes* var. *aureus* effected by quaternaries B and C were similar, only a few bacteria per milliliter being present after an exposure of 300 sec. However, the numbers of *E. coli* were many times greater for all periods of exposure in cases where quaternary C was used, and at 300 sec. the numbers of surviving bacteria with quaternary C were approximately 1,000 times greater than with quaternary B.

To determine the differences caused by using congo red in checking the numbers of bacteria present in milk cans after treatment with a quaternary, 10-gal. milk cans were washed with a non-ionic detergent and sanitized by an atomized mixture of steam and 800 ppm. of quaternary D in a can washer. The mixture of quaternary and condensed steam has been calculated to contain about 200 ppm. quaternary D. Cans were rinsed either with sterile distilled water or with sterile distilled water containing 80 ppm. of congo red. The results of plate counts were higher in cases where congo red was included in the can rinse water. The average plate count of 20 cans with which congo red was not used was 350 per can, and only two gave counts as high as 1,000 per can. The 50 cans with which congo red was used to neutralize residual quaternary gave an average plate count of 1,720 per can; 11 cans had counts of 1,000 or more.

DISCUSSION

Exposure of *Escherichia coli* 198 and *Micrococcus pyogenes* var. *aureus 209* to concentrations of congo red as great as 400 ppm. for 2 hr. was not found to decrease significantly the numbers of test bacteria, and the inclusion of 150 ppm. of congo red in a broth medium resulted in the development of turbidity by *E. coli* after 60-min. exposure to 50 and 100 ppm. of quaternary A (alkenyl dimethyl benzyl ammonium chloride). Prior to the addition of congo red to the broth, the development of turbidity was irregular and in many cases did not occur after exposure periods of only 2 min. Congo red seemed to permit growth in the subculture tubes by neutralizing the quaternary carried over with the inoculum from the exposure tube.

Use of 50 or 200 ppm. congo red in the dilution blanks used for determining the numbers of *M. pyogenes* var. *aureus* which survived treatment with 50 ppm. of quaternary B (alkyl dimethyl benzyl ammonium chloride) resulted in slightly greater numbers of surviving bacteria than did use of 500 ppm. of lecithin (table 1). Furthermore, the numbers of surviving bacteria with 5 ppm. of congo red were less than with the higher concentrations of congo red or with lecithin. Thus, a ratio of congo red to quaternary of 1: 1 did not seem to prevent adequately the action of quaternary on bacteria in the dilution blanks. There were no appreciable differences between the numbers of bacteria which survived ratios of congo red to quaternary of 10: 1 and 40: 1, and a ratio of 10: 1 or greater seemingly provided sufficient congo red for rapid neutralization of the quaternary.

The relatively small decreases in numbers of surviving bacteria in cases where bacteria were added to mixtures of inhibitor and quaternary (table 2) indicated the effectiveness of congo red as a neutralizer for quaternary B. With the use

of either congo red or lecithin as an inhibitor following exposure of bacteria to quaternary (table 1), the numbers of surviving bacteria decreased rapidly. This seemed to indicate that the inhibitors only neutralized residual quaternary and did not affect bacteria which already had been inactivated by the quaternary.

Quaternary C (N (acyl colamino formyl methyl) pyridinium chloride) seemed to reduce the numbers of *M. pyogenes* var. *aureus* as rapidly as did quaternary B, but quaternary C was not nearly as active as quaternary B against *E. coli*. This work substantiates that of several investigators who have indicated that great care should be taken in adopting quaternaries of unknown sanitizing value. Baker *et al.* (1) indicated that the relatively high bactericidal properties of quaternaries of the type of quaternary B may be due in part to the presence of a benzyl group.

The addition of congo red to rinse water used in determining the numbers of bacteria present in milk cans immediately after sanitizing with quaternary D (methyl dodecyl benzyl trimethyl ammonium chloride) resulted in higher counts than in cases in which no inhibitor was used. This indicated that a solution of congo red is superior to distilled water, but it did not indicate that congo red is superior to other inhibitors which might have been used. Equipment sanitized by the above method contains residual quaternary which may impart germicidal and prolonged bacteriostatic effect. However, the use of an inhibitor for quaternaries, similar in effect to the use of sodium thiosulfate with chlorine, would seem useful in determining the sanitary condition of equipment by permitting the results obtained to represent the sanitary condition at the time of sampling. A comparison between congo red and compounds such as lecithin and other proposed inhibitors for quaternaries, buffered distilled water with and without the addition of congo red or other inhibitor, and sterile milk would be of interest. A diluent might be devised which would result in greater numbers of surviving bacteria than was the case in this experiment.

Congo red seems to have possibilities for use as an inhibitor for quaternaries. It is a readily-soluble, synthetic, certified acidic dye of definite chemical structure and possesses the additional feature of imparting to colonies a red coloration which aids in their enumeration. However, inasmuch as only two different bacterial species were used, in order to recommend congo red unreservedly the absence of significant toxic effect by the maximum amounts of congo red to be employed would have to be established experimentally for many types of bacteria. It also would be necessary to establish the inhibitory action of congo red against a larger number of quaternaries and over a sufficiently wide range of pH to include those encountered in studying the germicidal action of quaternary compounds. Possibly it would be necessary to buffer solutions of inhibitor to compensate for differences in pH of germicidal solutions. In this work inhibitors and glucose were omitted from the plating medium. Although the action of congo red on the quaternaries used seemed extremely rapid, the presence of the inhibitors in the plating medium might have permitted even greater recovery of bacteria after treatment with quaternary. Also, the presence of glucose in the plating medium might have permitted the growth of weakened bacteria which were unable to form colonies in the absence of fermentable carbohydrate.

SUMMARY

Congo red, a synthetic, certified acidic dye, showed possibilities for application as a neutralizing agent for quaternary ammonium compounds.

A ratio of congo red to quaternary of 10:1 seemed as satisfactory as higher concentrations of the inhibitor. Congo red, in concentrations as great as 400 ppm. and during incubation periods as long as 2 hr., was not found to be significantly toxic to the two types of bacteria used.

A comparison of congo red and lecithin as inhibitors for quaternary ammonium compounds indicated that congo red was slightly more effective than lecithin. The action of this anionic dye as an inhibitor for cationic compounds appeared to be that of neutralizing residual quaternary, thus preventing further action on bacteria.

The use of congo red as a quaternary neutralizer after sanitization of milk cans with a quaternary resulted in the recovery of larger numbers of viable bacteria per can than in cases where the dye was not used.

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THE RELATIONSHIP BETWEEN CERTAIN SEMEN QUALITY TESTS AND FERTILITY AND THE INTERRELATIONSHIP OF THESE TESTS

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One of the paramount problems in the artificial breeding of dairy cattle is that of evaluating the quality and potential fertilizing capacity of semen. The most popular and widely used semen quality tests are (a) the microscopic estimation of motility (1, 3, 4, 5, 6, 8, 10, 15, 19, 20, 21, 22, 23, 25, 26), (b) the enumeration of spermatozoa (concentration) (1, 3, 4 ,8, 12, 15, 19, 20, 21, 23, 24) and (c) the methylene blue reduction test (2, 3, 4, 7, 8, 12, 15, 23). The Cornell investigators (2, 3, 4, 12, 13, 14, 15, 16, 17) have established minimum standards for all semen that is to be used for breeding purposes. These standards are as follows: A concentration of at least 900 million spermatozoa per milliliter of semen, an initial motility of 70 per cent or better and a methylene blue reduction time of 9 min. or less. Evaluating semen on this basis usually has resulted in the discarding of approximately one-third of the ejaculates from highly fertile bulls and a conception rate of about 60 per cent (based on 60- to 90-day non-returns to first services.

Less popular semen quality tests are the cold shock test (8, 10, 19, 20, 22), incubation test (2, 8), livability or viability (2, 20, 21, 22) and the resistance tests (1). The latter test measures the resistance of spermatozoa to high concentrations (volumes) of a 1 per cent NaCl solution. This test apparently has been used rather widely in Russia, but it has not been used to any extent in this country.

A review of the literature reveals that there is much controversy as to the reliability and practical applications of the above tests. However, there is general agreement that no single test appears to be highly reliable. Even combinations of the above tests are not always reliable. Stone *et al.* (19) reported on the relationships of some of these tests as well as live spermatozoa relationship and fertility of dairy bull semen. No study has been reported on the comparison of the accuracy with which all of the above mentioned tests conducted simultaneously on the same semen samples predict semen quality and fertility. Also, some of the tests have not been modified or improved since their discovery.

The purposes of the investigations reported herein were as follows: (a) to modify the methylene blue reduction test, (b) to compare its accuracy with that of initial motility, concentration, conventional methylene blue reduction test, duration of motility in storage at 40° F. and at high temperatures, and the resistance test in measuring or predicting semen quality and fertility, (c) to de-

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termine the interrelationship of these tests and (d) to determine the importance of the numbers of motile spermatozoa per insemination.

EXPERIMENTAL METHODS AND MATERIALS

Concentration. Spermatozoa concentration was determined by the use of a Klett-Summerson photoelectric colorimeter which previously had been calibrated by determining the colorimetric density readings and hemocytometer counts on 100 ejaculates (15).

Methylene blue reduction tests. The conventional methylene blue reduction test (hereafter referred to as methylene blue reduction test I) was conducted according to the method described by Beck and Salisbury (2, 8). In this test the extension or dilution of the semen is by volume, 0.2 ml. of semen to 0.8 ml. of egg yolk-citrate extender. Therefore, for the average ejaculate about 240 million spermatozoa would be present.

In methylene blue reduction test II the numbers of spermatozoa were standardized to 240 million. Sufficient volumes of semen to contain this number of spermatozoa were added to a 10×75 mm. test tube and then enough egg yolk-citrate extender was added to bring the total volume to 1.0 ml. The remainder of the test was the same as test I. Methylene blue reduction test III was the same as test II except that 300 million spermatozoa were used.

Other tests. Initial motility (2, 3, 4, 8), incubation test (2, 8), cold shock test (8, 10, 19, 20, 22) and the resistance test (1) were conducted as has been described previously in the literature with some alterations. For the incubation test the semen was extended 1:4 with egg yolk-citrate-sulfanilamide and placed in a hot water bath at 115° F. for 30 min. The semen for the cold shock test also was extended at the rate of 1:4. The livability data were obtained in the form of percentages of motile spermatozoa in subsamples of semen extended and shipped for breeding purposes at the end of 3 days of storage at 40° F.

Semen used for shipment. The following criteria were used to evaluate the semen for shipment: initial motility, concentration and the methylene blue reduction tests as described above. Semen samples which contained at least 50 per cent motile spermatozoa and a minimum of 500 million spermatozoa per milliliter and which had a methylene blue reduction time of not over 9 min. for any of the three tests described above were extended with egg yolk-citrate-sulfanilamide at an average rate of 1:77, ranging from 1:29 to 1:125. The citrate buffer used contained 3.0 per cent sodium citrate dihydrate and 0.6 per cent sulfanilamide. It was mixed with equal portions of egg yolk to form the extender. The extended samples then were shipped to breeding technicians of the Louisiana Artificial Breeding Cooperative, Inc., for routine inseminations during January, February and March, 1949.

Measurement of results. The fertility data were based on per cent 60- to 90-day non-returns to first services. The simple, partial and multiple correlation coefficients reported herein were calculated according to the methods described by Love (11) and Snedecor (18).

RESULTS AND DISCUSSION

Semen characteristics and fertility. A summary of the characteristics of the semen used in this study is shown in table 1. It will be noted that most of the 100 semen samples used were of high quality.

TABLE 1

Summary of semen characteristics

	Mean	Standard error	Range
Initial motility (%)	63.3	0.86	50 – 80
Concentration (*millions/ml.*)	1,399	53.7	488 – 2,259
M.B.R.T.[a]— I (*min.*)	5.76	0.32	2.5– 30.0
M.B.R.T. — II (*min.*)	7.46	0.26	4.0– 20.0
M.B.R.T. —III (*min.*)	5.96	0.18	3.0– 12.0
Motility at end of 3 d. (%)	55.3	1.02	20 – 70
Motility after cold shock	52.9	1.37	20 – 70
Resistance (*R. values*)	2,914	63	500 –12,000

[a] M.B.R.T. = Methylene blue reduction time.

Table 2 shows in summary form the range in number of semen samples used per bull, number of first services and per cent 60- to 90-day non-returns, as well as the totals.

Correlations and relationships. Table 3 presents the simple correlation coefficients between the various semen quality tests and fertility, as well as certain partial and multiple correlation coefficients. These coefficients are based on results from all of the 100 semen samples, a "total" basis.

It will be noted from table 3 that concentration of the individual ejaculates was highly significantly correlated with fertility ($r = +0.2834$). When the means for the individual bulls were used for the analysis, a more highly significant correlation coefficient ($r = +0.5678$) was obtained. Examination of the data revealed that fertility increased with each increase in concentration of non-extended semen. Furthermore, it was found that the numbers of motile spermatozoa per milliliter of extended semen or per insemination were highly significantly correlated with fertility ($r = +0.4912$). It also should be mentioned that the "within bull" correlation coefficient was highly significant ($r = +0.3637$). However, the "between bull" correlation coefficient was not significant ($r = +0.0990$). These results indicate that the extension of semen should be on the basis of numbers of motile spermatozoa rather than by volume, as has been reported previously by several investigators (13, 14, 16, 24).

TABLE 2

Summary of fertility data

	No. of semen samples	No. of 1st services	Per cent 60- to 90-day non-returns
Range for bulls	1–9	14–435	40.9–88.2
Totals	100	2,985	57.8

Initial motility was not significantly correlated with fertility, ($r = +0.0472$, table 3). However, since only samples containing at least 50 per cent progressively motile spermatozoa were used, this probably should have been expected. When the semen samples were arranged in 50, 60, 70 and 80 per cent initial motility groups, their fertility was 55.8, 57.5, 58.4 and 58.6 per cent, respectively.

Highly significant negative correlation coefficients of -0.6675, -0.3147, -0.3790 and -0.3415, as shown in table 3, were obtained between M.B.R.T.–I and concentration, initial motility, motility after cold shock and motility at the end of 3 days of storage at 40° F., respectively. The correlations between M.B.R.T.–I (conventional test) and concentration and initial motility were in agreement with those reported by Beck and Salisbury (2) and by VanDemark *et al.* (23). It was evident in this study that a large portion of the variation in the M.B.R.T.–I was due to the concentration and to the initial motility of the spermatozoa of the semen samples, with a larger portion being due to the former. This is made more obvious by the partial correlation coefficients given in table 3. The partial correlation coefficient between M.B.R.T.–I (x_3) and initial motility (x_1) independent of concentration (x_2) was -0.5003, as compared to that of -0.7339 between the same methylene blue reduction times and concentration independent of initial motility.

Since a larger portion of the variation in the conventional methylene blue reduction time (M.B.R.T.–I) had been observed prior to this study to be due to concentration than to initial motility, it was believed that by standardizing the numbers of spermatozoa in this test a more accurate test check could be made on the microscopic estimations of initial motility which are subject to much human error. Another reason for the standardization was that concentration was being determined routinely anyway. It is, therefore, of interest to observe that the correlation coefficients between M.B.R.T.–II and initial motility, motility after cold shock and motility at the end of 3 days of storage were -0.8123, -0.2919 and -0.5038, respectively. Correlation coefficients between M.B.R.T.–III and the same tests were -0.8638, -0.2527 and -0.8546, respectively. All of these correlations were highly significant statistically. Therefore, correlations between the modified methylene blue reduction times and initial motility were increased greatly over that between the conventional test and initial motility.

The only methylene blue reduction time significantly correlated with fertility was M.B.R.T.–III (300 million spermatozoa) ($r = -0.2150$). This probably should have been expected, since only high quality semen was used. However, each of the methylene blue reduction tests showed a definite negative relationship with fertility when the data were grouped according to different time intervals. On this basis, the samples with the shortest reduction times were the highest in fertility. It appears from the simple correlation coefficients as presented in table 3 and the grouped data that either of the modified tests would show improvement over the conventional test. This is made more evident when the multiple correlation coefficients in table 3 are observed. The multiple correlation coefficient between initial motility, concentration, M.B.R.T.–I and fertility was 0.2697 and was not significant; in contrast, M.B.R.T.–III gave a multiple correlation co-

TABLE 3

Simple, partial and multiple correlation coefficients between certain tests for estimating semen quality and fertility and other semen characteristics

x's and y's	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}
Initial motility—x_1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Concentration—x_2	− 0.0845	.....	.....	.....	.....	.....	.....	.....	.....	.....
M.B.R.T.—I—x_3	− 0.3147**	− 0.6675**	.....	.....	.....	.....	.....	.....	.....	.....
M.B.R.T.—II—x_4	− 0.8123**	+ 0.0260	.....	.....	.....	.....	.....	.....	.....	.....
M.B.R.T.—III—x_5	− 0.8636**	+ 0.0931	.....	.....	.....	.....	.....	.....	.....	.....
Motility at end of 3-d.-storage—x_6	.....	− 0.1697	− 0.3416**	− 0.5038**	− 0.8546**	.....	.....	.....	.....	.....
Motility after cold shock—x_7	− 0.5185**	.....	− 0.3790**	− 0.2919**	− 0.2727**	− 0.8177**	.....	.....	.....	.....
Resistance to NaCl solution—x_8	− 0.0440	+ 0.0519	.....	.....	.....	+ 0.0201	.....	.....	.....	.....
No. motile sperm per ml. (non-extended semen)—x_9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
No. motile sperm per insemination—x_{10}	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fertility—y	+ 0.0472	+ 0.2834**	− 0.0951	− 0.1162	− 0.2150*	+ 0.0453	− 0.0577	+ 0.0314	+ 0.2551*	+ 0.4912**

$r_{x_2x_3 \cdot x_1} = -0.7339^{**}$ $\quad R_{x_1x_2x_3y} = 0.2697$ $\quad R_{x_1x_3y} = 0.1251$ $\quad R_{x_1x_5y} = 0.5095^{**}$

$r_{x_1x_3 \cdot x_2} = -0.5003^{**}$ $\quad R_{x_1x_2x_5y} = 0.3522^{**}$ $\quad R_{x_1x_4y} = 0.5020^{**}$

* Significant at 5% level of probability.
** Significant at 1% level of probability.

efficient of 0.3522 which was highly significant statistically with the same items. Both of the modified tests can be conducted easily and rapidly when the concentration of a given ejaculate is known. However, test III had the advantage of being more rapid and being more highly correlated with initial motility ($r = -0.8636$), motility at the end of 3 days of storage ($r = -0.8177$) and fertility ($r = -0.2150$) than test II.

It will be noted in table 3 that the Russian resistance test (resistance of spermatozoa to a 1 per cent NaCl solution) was not significantly correlated with any of the other tests or with fertility. It also should be mentioned that this test is time consuming and not a practical or easy test to conduct.

The accuracy and reliability of the duration of motility at 115° F. for 30 min. in semen extended 1:4 with egg yolk-citrate-sulfanilamide as a test for semen quality and fertility are questionable. Under the conditions of this experiment, all motility ceased in 45 of the 100 samples during the incubation. Consequently, no correlations were calculated for this test.

SUMMARY AND CONCLUSIONS

Based on the results of this experiment in which simple, partial and multiple correlation analyses of the semen quality and fertility data on 100 semen samples were used and on a review of the literature, the following conclusions appear to be justified:

No single rapid test has been devised that will accurately and reliably measure or predict semen quality and fertility.

Concentration, particularly when semen is extended at high ratios, is a very important test.

The modified methylene blue reduction test, especially the test in which numbers of spermatozoa for all ejaculates are standardized at 300 million per milliliter, will more accurately measure initial spermatozoan activity (motility), viability and fertility than the conventional test.

The resistance and incubation tests are impractical and unreliable.

The following combination of tests and minimum standards are recommended for evaluating bull semen: (a) An initial progressive motility of at least 50 per cent. (b) The concentration as determined by hemocytometer, photoelectric colorimeter or opacity standard method should be at least 500 million spermatozoa per milliliter. (c) The modified methylene blue reduction time, using 300 million spermatozoa per milliliter, should not exceed 9 min., and preferably 7 min.

Bull semen should be extended on the basis of numbers of progressively motile spermatozoa rather than by volume, probably not less than 12 to 15 million motile spermatozoa per milliliter.

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TURBIDITY AS A MEANS FOR DETERMINING THE EFFICIENCY OF HOMOGENIZATION[1]

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It is well known that homogenization modifies the appearance of milk. The breaking up of the fat globules causes them to scatter a greater amount of light and gives the milk a more opaque appearance. It has been found that a rough estimation of the total solids of milk can be made by diluting the milk to the range of turbidity which can be measured in a photoelectric colorimeter (1). The turbidity per unit concentration of total solids varies with the fat content of the milk and also with the efficiency of homogenization since about three-fourths of the total turbidity can be attributed to the fat phase. When the turbidity due to the non-fat portion of the milk has been reduced to a negligible quantity by the addition of ammonia, the turbidity of the fat globules per unit concentration of fat is shown to be closely related to the particle size distribution. Since Leviton and Haller (3) have suggested that the fat content of milk might be estimated by a turbidimetric method, they must assume a constant particle size distribution.

The test presented here has been developed by using fresh milk and evaporated milk. However, it has been modified for use with powdered whole milk and found to be quite satisfactory. No attempt has been made to apply the test to ice cream mix.

PROCEDURE

After gently but thoroughly mixing the milk, 1.00 ml. was measured into a 500-ml. Erlenmeyer flask. To this was added 5 ml. of 5*N* NH_4OH, and the mixture was shaken. Then 250 ml. of water at 50–55° C. were added. After mixing gently by rotation and allowing to stand 30 min., the mixture was rotated again and poured into colorimeter tubes. These were cooled to 30° C. and the turbidity measured.

The Evelyn photoelectric colorimeter equipped with a 515 mμ filter has been used to measure the turbidity. The instrument was adjusted to 100 per cent transmission with water and the transmission of the unknowns determined. By a modified Babcock determination (4) of fat in the original milk, the fat concentration in the final dilution was estimated as milligrams of fat per milliliter. From the per cent light transmission the corresponding optical density, or "*L* value," was found. The ratio of the *L* value to the mg. of fat per ml. in the final dilution gave the turbidity per unit concentration, or "*K* value." When unhomogenized milk was treated as above, a *K* value of approximately 1.0 was

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found. The K value increased with the efficiency of homogenization, a value of 2.2 showing proper homogenization as defined by Doan and Mykleby (2).

It sometimes is difficult to reconstitute whole milk powder adequately for homogenization efficiency tests. In such cases the best procedure found was to weigh out accurately on an analytical balance 140–180 mg. of the whole milk powder to be placed in a 500-ml. Erlenmeyer flask. With the addition of 250 ml. of 0.1N NH_4OH at 50° C., the powder may be completely dissolved by gentle intermittent rotation of the flask. The rest of the test is conducted as with fresh milk. The concentration of fat is calculated from the known weight of powder and its percentage fat.

EXPERIMENTAL RESULTS

In applying the test to reconstituted whole milk powder, the average reproducibility is about 2 per cent of the value for K. No appreciable effect on the turbidity was found when the temperature of the final diluting water varied a few degrees. Also, there was no appreciable change when the colorimeter tubes were allowed to stand for 30 min. after the first reading. However, each person who plans to use the test should study under his own conditions the reproducibility and effect of slight variations in the procedure.

One means of testing the method was to mix properly homogenized milk in definite proportions with other portions of the same milk which had not been run through the homogenizer. The milk used for this purpose was pasteurized at 170° F. for 10 min., then passed three times through a Manton Gaulin laboratory model C. G. B. homogenizer, the first stage being set at 3000 and the second stage at 500 lb. pressure. The turbidity test was run on the mixed milks with the results shown in fig. 1 (right hand curve). It will be seen that there is a proportionate increase in the value of K with the amount of homogenized milk added.

Another means of testing the method was to homogenize different portions of a batch of milk with increasing pressure on the first stage. The effect on the corresponding turbidity values are shown on the left in fig. 1. There is a leveling off in the value of K at the higher homogenization pressures, which is the typical shape for these curves.

The turbidities of homogenized samples were compared also with the cream rising efficiency test. The milk was stored for 48 hr. at 40° F. in pint bottles. The top 50-ml. portion then was removed, using a siphon-opening-up arrangement as described by Doan and Mykleby (2). The index was calculated as the ratio of the difference between the per cent fat in the top layer and the per cent fat in the remainder of the bottle to the per cent fat found in the top layer and calculated in per cent. Figure 2 shows the results. Here, again, a straight line relationship shows the proportionality between the cream rising index and the turbidity test K value. If a K value of 2.2 is considered as the lower limit for properly homogenized milk it can be seen to correspond to a little under the 10 per cent rising index used as a limit by the U. S. Public Health Service.

The content of fat in the milk seems to have no effect on the test in the range below 5 per cent fat, providing the fat content is known. When whole milk is

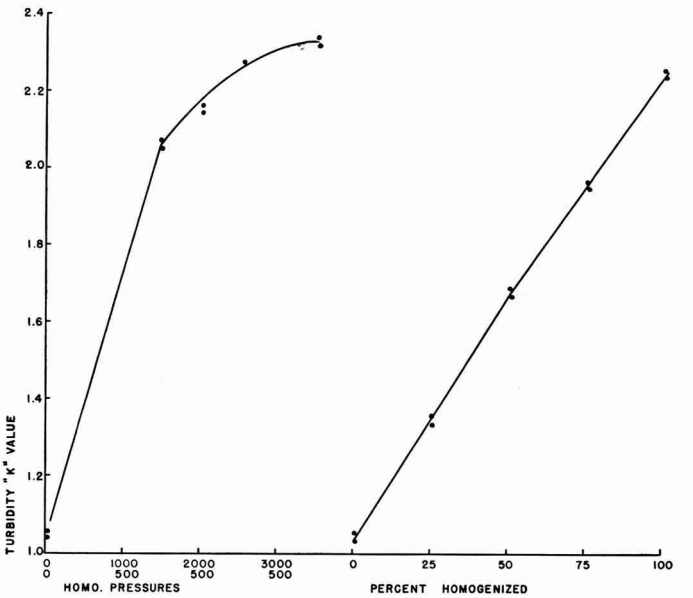

FIG. 1. Relation of the turbidity constant of the fat to the homogenization treatment.

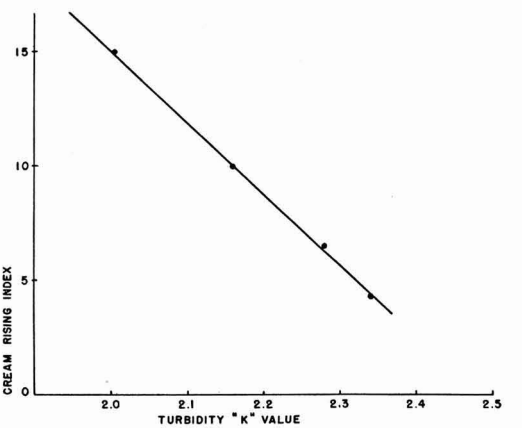

FIG. 2. The linear relation between the U. S. Public Health Service cream rising index and the turbidity constant.

diluted with separated milk the K value remains constant. In fact, knowing the K value one can easily estimate the fat content of the sample from the turbidity,

as suggested by Leviton and Haller (3). This appears to be a practical method for measuring the percentage of fat in the lower range of fat concentration where the ordinary Babcock method is not suitable.

When the method was applied to separated milk it was necessary to decrease the extent of dilution in order that sufficient turbidity be developed for measurement. Under these conditions the milk proteins contributed a greater share of the turbidity and it was found necessary to correct for them.

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VARIATIONS IN HEART RATE OF DAIRY COWS

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Detailed data on the various factors that affect the heart rate of dairy cows are very limited. Most physiology textbooks, as for example Dukes (6), list size and age of animal, excitement, exercise, external temperature, digestion, sleep and pathological conditions as factors affecting heart rate. The quantitative effects of these factors have been studied incompletely or not at all in dairy cows.

Blaxter (3, 5) has made an excellent study of normal physiological variations in heart rate on a limited number of cows, and Ritzman and Benedict (10) have published observations on steers. Neither of these studies appears to have been mentioned in physiology textbooks or generally to be known by research investigators. Data published by Fuller (7) indicate the minimum and maximum rates were 38 and 96 heart beats per minute, with an average of 66 beats. The rates noted by Alfredson and Sykes (1) are in agreement with those found by Fuller, but they also point out that dairy calves under 18 mo. have a higher heart rate than animals above this age.

This study was made to check and enlarge upon the published data and to show the natural variation in heart rate and how it is influenced by feed intake and stage of lactation. An abstract covering most of this work has been published previously (13).

PROCEDURE

Heart rates were obtained with a stethoscope placed on the left chest wall in the region of the heart. This method produced an immediate but transient increase in heart rate of cows not accustomed to this procedure. After a period of 0.5 to 2 min., however, the heart rate decreased and became steady. This phenomenon also was noted by Blaxter (3). When four consecutive 15-sec. periods of auscultation gave identical heart rates, this value was taken as the true heart rate. When heart rates were taken weekly on cows for a long period of time (6 mo. to 3 yr.) there was very little excitation and true rates were obtained on the initial observations with most of these cows.

Palpation of surface arteries was unsatisfactory, as compared to using the stethoscope, for this investigation and for others (2, 3, 5, 13, 14).

All heart rates were taken at 10 to 11 a.m., when the cows were standing in the stanchion and not ruminating. This was at least 3 hr. after the morning hay feeding and 5 hr. after the morning grain feeding. This procedure eliminated the variable but definite increase in heart rate due to variations in body position, act of rumination and ingestion of feed that had been noted by others (2, 3) and confirmed during these observations. The heart rates obtained could be classified as standing-resting heart rates. In obtaining heart rates, precau-

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tions must be taken to avoid excitement and to adhere strictly to standardized conditions in order to obtain rates unaffected by factors other than those being studied.

All cows used in these experiments were barn fed. The ration consisted of alfalfa and timothy hays, corn silage and a grain mixture (14). Weighed amounts of each were fed and refusals of each were weighed daily. This gave an accurate record from which the total digestible nutrient (T.D.N.) intake was calculated by using Morrison's coefficients (8).

The cows used in these observations and experiments all were normal dairy cows of the Jersey and Holstein breeds. All cows received normal rations and care, except a few cows that received thyroprotein. The latter are specifically mentioned in the text and in table 3. The food intake was at or near Morrison's maximum requirement (8) unless otherwise noted in the text. The heart rate of some cows was observed over a period of three lactations, but for most cows the period of observation was only 13 mo.

For presentation here, heart rates have been averaged by the time intervals given in the tables and graphs. In most cases the heart rates were determined once a week, but in some cases they were determined twice a week and in other cases every other week.

RESULTS AND DISCUSSION

The average heart rates of normal cows fed at requirement through 325 days of lactation are presented in table 1, as averaged by 25-day periods during the

TABLE 1

Heart rate of dairy cows during lactation (beats/min.)

Days in lactation	1st lactation av.	2d to 7th lactation av.	1st to 7th lactation av.	Range Min.–Max.
1– 25	79.8 (15)*	74.2 (20)	76.6 (35)	58–96
26– 50	78.2 (17)	74.6 (18)	76.4 (35)	60–90
51– 75	72.6 (5)	69.1 (15)	70.0 (20)	60–80
76–100	72.6 (5)	67.4 (12)	68.9 (17)	60–76
101–125	70.8 (6)	63.8 (12)	66.1 (18)	58–76
126–150	68.0 (7)	65.6 (12)	66.5 (19)	54–80
151–175	66.5 (6)	64.2 (13)	64.9 (19)	52–80
176–200	64.2 (5)	62.8 (13)	63.2 (18)	48–80
201–225	69.2 (5)	62.9 (13)	64.7 (18)	44–80
226–250	64.5 (4)	63.8 (13)	63.9 (17)	48–76
251–275	55.0 (3)	62.3 (12)	60.9 (15)	48–72
276–300	56.0 (3)	61.8 (12)	60.0 (15)	48–72
301–325	57.0 (3)	62.7 (10)	62.2 (13)	44–72

* (Figures in parentheses indicate the number of cows used in obtaining the average figure given.)

lactation. The number of cows included in each 25-day average is given in parentheses and the minimum and maximum values observed also are given in table 1. The total number of heart rate determinations involved was 552.

There was a large variation in heart rate between individual cows at comparable stages of lactation. The cows that had the slower heart rates during early lactation tended to have the slower rates during late lactation. Similarly,

the cows with the fastest heart rates during early lactation remained in the group having faster rates at all periods during lactation. All the minimum heart rates shown in table 1 originated from only seven cows, and one of these cows was represented in eight of the 13 minimum values found. All the maximum values originated from ten other cows, and three of these cows each had three of the maximum values shown in table 1.

Even with the between-cow variation noted, all cows followed a definite trend downward in heart rate as the lactation period progressed. The graph depicting heart rate during lactation is similar to an average milk production curve. The authors previously have commented on this similarity (13, 14).

The variability in heart rate is greater during the first 50 days of lactation than at later stages of lactation. Also, the relation between heart rate and milk production is probably less during the first 50 days than after this time. By discarding the values found during the first 50 days and plotting the logarithm of heart rate from 50 to 300 days vs. days in lactation, a straight-line relationship was obtained which had the following formula: Logarithm of heart rate = $1.858451 - 0.00026631\ X$. When all values during the 300 days in lactation are considered, the formula found was: Logarithm of heart rate = $1.87702 - 0.00035467\ X$. Both regression coefficients were highly significant ($P > 0.99$) (11).

The heart rate of the heifers during their first lactation was somewhat higher than the rate found for cows in their second to seventh lactations (table 1). The difference was about four beats per minute, when comparisons were made with adequate numbers of animals. Blaxter (4) noted a difference of six beats per minute between heifers and old cows. The low rates for the first-lactation heifers after 250 days in lactation may be explained by the fact that there are only three animals represented in each period and two of these three cows had the minimum and next to minimum values found for the last three 25-day lactation periods given in table 1.

The effect of late pregnancy on heart rate is shown in table 2. The data

TABLE 2

Heart rate of dairy cows during the last 3 mo. of pregnancy (beats/min.)

Days before calving	Range Min.–Max.	Av. heart rate
1–10	80–116	91.7 (9)
11–20	76– 88	80.4 (12)
21–30	64–100	77.6 (10)
31–40	60– 84	71.3 (14)
41–50	60– 80	71.5 (8)
51–60	56– 80	68.7 (11)
61–70	56– 84	67.4 (13)
71–80	52– 77	66.6 (8)
81–90	50– 72	64.3 (11)

represent 119 heart rate determinations on 19 cows averaged by 10-day periods preceding parturition. The range of rates observed was large, but there was a very definite trend toward a higher heart rate as calving time was approached.

This increase was rapid during the last 6 wk. of pregnancy. The small increase in average heart rate noted in table 1 for the last 25-day period (301–325 days in lactation) is due to the fact that some of the cows were within 2 mo. of their next parturition.

TABLE 3

Heart rate of cows fed two different levels of T.D.N. during consecutive lactations (beats/min.)

Interval in lactation	Cow no. and lactation							Av. all cows
	261–2	485–2	604–1	614–1	268–1	269–1	487–1	
(*d.*)				TDN—100%				
1– 25	67	72	81	90	–	–	–	77.5
26– 50	67	80	75	–	–	–	–	74.0
Thyroprotein added	–	–	+	+	+	+	+	
51– 75	63	71	76	79	90	–	89	78.0
76–100	64	67	74	76	75	85	89	75.7
101–125	62	59	71	72	70	66	81	68.7
126–150	62	56	72	–	73	71	70	67.3
151–175	61	52	58	60	70	71	75	63.9
176–200	60	50	58	64	64	63	70	61.3
201–225	56	46	51	–	60	60	63	56.0
226–250	55	50	57	–	57	69	55	57.2
251–275	55	52	55	–	52	59	54	54.5
276–300	60	52	55	–	53	54	58	55.3
Av. 50–300 days	59.8	55.5	62.7	66.4	66.4	66.4	70.4	64.0
TDN consumed as % of requirement 50–300 days	99.7	100.8	104.4	98.4	114.2	113.1	107.1	105.3
F.C.M.	8,879	8,657	5,561	5,689	6,951	4,737	7,385	6,746[a]
	261–3	485–3	604–2	614–2	268–2	269–2	487–2	
				TDN—100%				
1– 25	76	84	68	86	–	–	–	78.5
26– 50	73	74	74	–	–	–	–	73.7
				TDN—125%				
Thyroprotein added	–	–	+	+	+	+	+	
51– 75	70	86	80	92	76	–	88	82.0
76–100	71	76	86	80	92	94	89	84.0
101–125	70	68	84	76	85	93	89	80.7
126–150	64	68	83	–	80	81	88	77.3
151–175	71	70	82	76	86	74	76	76.4
176–200	68	75	68	64	86	77	70	72.6
201–225	66	80	71	–	79	73	73	73.7
226–250	74	72	72	–	75	74	64	71.8
251–275	65	64	76	–	77	64	65	68.5
276–300	62	68	66	–	64	62	61	63.8
Av. 50–300 days	68.1	72.7	76.8	77.6	80.0	76.9	76.3	75.0
TDN consumed as % of requirement 50–300 days	110.2	122.2	112.8	117.8	125.5	119.0	132.3	119.9
F.C.M	10,564	9,449	7,301	5,678	8,068	6,900	5,405	7,993[a]

[a] Cow 487 injured two teats during second lactation. F.C.M. average is for 6 other cows.

In order to show the effect of feed intake on heart rate, the following com-

parison was made with seven cows. All seven cows were fed 100 per cent of their T.D.N. requirement throughout one lactation and during the first 50 days of the next lactation, and thereafter they were fed 125 per cent of their requirement. Five of the seven cows also received thyroprotein starting on the 50th day of each lactation period and continued until 90 days prepartum. The average heart rates and actual levels of feed consumption during the 50th to 300th day of the two consecutive lactations are given in table 3. The milk production during this period also is given in table 3, expressed as 4 per cent fat-corrected milk (F.C.M.) on a mature equivalent basis. The average heart rate of the seven cows during the period of extra T.D.N. feeding, when the cows actually consumed 120 per cent of requirement, was about 11 beats per minute faster than when average feed intake was 105 per cent of requirement during the preceding lactation. This relationship was the same for the five cows that were fed thyroprotein as for the two cows not fed thyroprotein. The milk record of cow 487 was eliminated from the average because of injury to two teats during the second lactation. Also, cow 614 had an attack of mastitis during her second lactation. The average increase in heart rate of the seven cows from one lac-

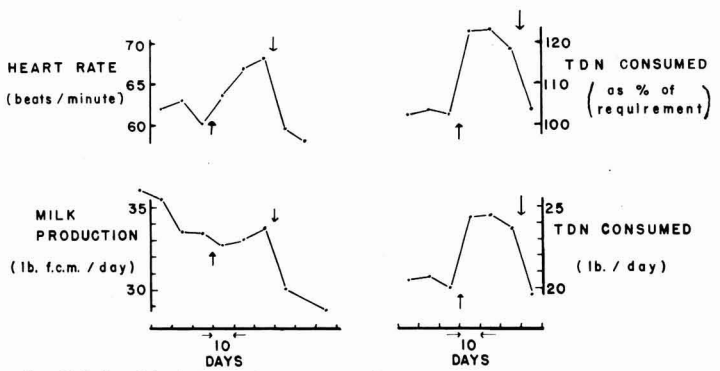

FIG. 1. Relationship between heart rate, milk production and total digestible nutrients (TDN) consumed. Data represent the average figures obtained on four dairy cows described in text. Arrows pointing upward indicate when extra TDN was offered and downward when extra TDN was withdrawn.

tation to the next was 17.7 per cent and of the six cows omitting 487 it was 19.9 per cent. The average increase in milk production of six cows during this period was 18.5 per cent.

A short experiment was carried out in order to more vividly illustrate the effect of T.D.N. intake on heart rate. Four cows which had been fed T.D.N. at 100 per cent of requirement were fed T.D.N. at 125 per cent of requirement for the next 30 days. The increase was made when the cows were 80, 80, 100 and 250 days postpartum. T.D.N. intake then was reduced back to 100 per cent but two of the cows were subsequently transferred to another experiment after 10 days of feeding at 100 per cent. The data are presented in figure 1. They show that the heart rate gradually increased when extra T.D.N. were consumed.

Also, milk production increased and showed a definite curve upwards instead of the normal downward trend.

When extra T.D.N. were removed, the heart rate showed an average decrease of 8.5 beats per minute during the first 10 days after reducing T.D.N. intake. This is in agreement with the results of Ritzman and Benedict (10). They noticed an average decrease of 10 beats per minute during the first week when steers were changed from maintenance to submaintenance ration (2). In contrast to the rapid drop when T.D.N. intake was reduced, the heart rate increased at a somewhat slower rate when T.D.N. was added. The heart rate was still increasing at the end of the 30-day period of extra T.D.N. feeding. This is in agreement with previous observations on steers where there was a continual but gradual increase in heart rate when changed to a fattening from a maintenance ration (2, 10).

In other work with steers (12), a change of 3.6 beats per minute was produced by each 10 per cent change (from 100 per cent of requirement) in feed consumption. In this short experiment with the four cows, the average heart rate increase during the 11th to 30th day of extra T.D.N. feeding was 7.2 beats per minute. During this time, the T.D.N. intake increased from 102.4 to 120.7 per cent, which gives an increase of 3.9 beats per 10 per cent change in feed consumption. This is in excellent agreement with the results on steers. However, a similar figure for the seven cows in table 3 with observations over 250 days was 7.7. This is a larger increase per unit change of feed than was observed on the shorter trials with cows or with steers. The fact that the heart rate of each of the four cows was constantly increasing during the 30-day period of extra T.D.N. feeding indicates that more than a 30-day observation is necessary in order to properly evaluate the effect of additional T.D.N. consumption on heart rate. Also, the maximum effect on heart rate shown by the data in table 3 did not occur until 25–50 days after initiating extra T.D.N. feeding.

Ralston and co-workers (9) reported that there was a seasonal variation in heart rate of dairy cows. Blaxter and Price (5) noted a similar phenomenon which they stated probably was of nutritional origin. Our data indicate that there is no seasonal effect, *per se,* on heart rate when T.D.N. intake is kept at requirement and calving effects are eliminated.

All heart rates obtained on normal cows fed at 100 per cent of requirement were tabulated for the months the above authors noted a seasonal variation, namely in April, June, August and October. The values obtained for cows within 60 days after and 90 days before calving were discarded. The average values found were 64, 64, 63 and 65 for the 4 mo., respectively. The seasonal effect reported by Ralston *et al.* (9) may have its origin in increased T.D.N. during the flush pasture season of April and October and/or increased incidence of calving effects during the spring and fall, or in other factors.

Another variation in heart rate of cows was an increase noted when the cows were in estrus. In 21 observations the heart rate before, during and after estrus averaged 73.6, 81.7 and 76.1 beats per minute, respectively. In another five observations it averaged 80.4 beats per minute during estrus and 72.0 at 2 to 3

days after estrus. Heart rates taken the week previous to or following estrus always were lower than when the cow was in estrus. Whether or not estrus *per se* or the accompanying excitement is the primary cause of this increase was not investigated. In our observations all the rates were taken when the animals were resting and had been confined to stanchions for at least 3 hr.

The effect of thyroprotein on heart rate is well known (9, 12, 13, 14) and its effect is noticeable in the values given in table 3.

During the course of this study, the articles by Blaxter (3) and by Blaxter and Price (5) came to the attention of the authors. The similarity of the results obtained in the present work with those obtained by Blaxter and Price is striking. Their studies (5) dealt for the most part with only four animals but were carried out in great detail. The pre-calving increase in heart rate observed in our cows averaged a little greater than that found in the four cows by Blaxter and Price (5). However, the extent of this increase is variable from cow to cow. The decrease in heart rate as lactation progresses was noted by Blaxter and Price, who also calculated that 71 per cent of the variation in milk yield was associated with variation in heart rate. They (5) indicated that heart rate could be influenced by level of feeding as did Ritzman and Benedict (2, 10) and Sykes *et al.* (12).

Ritzman and Benedict (2, 10) pointed out that the heart rate of an animal must be considered in relation to its metabolic level. This is further emphasized by the work of Blaxter (3, 5) and the work reported in this paper. This poses the question of what proportion of the variability in heart rate caused by body size (species variation) is due to differences in rates of metabolism. In this case, one can only refer to total metabolism for, as Blaxter and Price (5) have suggested, heart rate is related to total metabolism—not basal—especially in reference to the functioning dairy cow. Our results add further support to this suggestion.

SUMMARY

Heart rates were observed on 42 cows for periods varying from 2 mo. to 3 yr. The heart rate of normally-fed cows showed an increase of about 30 beats per minute during the last 3 mo. of gestation. During lactation, there was a gradual decrease in heart rate, which was similar to the milk-production decrease.

When nutrients were consumed at a level above requirement there was an increase in the heart rate. During short-time trials this increase was about 3.9 beats per minute for each 10 per cent increase in T.D.N. consumed above requirement. In studies over a complete lactation this figure was about 7.7. The increase in heart rate was not immediate but gradual when extra T.D.N. were fed, the maximum was not reached for about 30 days after increased feed intake occurred. However, when a decrease in T.D.N. consumption occurs there is a large and immediate decrease in heart rate. Heart rate is in direct proportion to the total metabolism of the dairy cow.

The results reported here show the importance of standardizing conditions and eliminating factors known to affect heart rate, in order to properly evaluate the results when determining heart rate.

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DEVELOPMENT OF THE BOVINE STOMACH DURING FETAL LIFE

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Development of the bovine stomach during fetal life is of interest as it may relate to early feeding possibilities, postnatal development and function. Growth of the bovine fetus as a unit was reported by Bergmann (2), Eckles (3), Hammond (4), Nichols (6), Swett *et al.* (7), Winters *et al.* (9) and by Becker and others (1). A microphotograph by Winters (9) showed the developing stomach in a 29-day fetus that weighed 0.28 g. Turner (8) studied the mammary gland, but little information has been published concerning growth of other organs in dairy calves during fetal life.

METHODS

Fetuses were obtained upon disposal of dairy cows from the station herd. Ages of fetuses were computed from the date of service, since the exact time of conception was unknown (5). Each fetus was weighed and the stomach removed. The organ was weighed intact in most instances, the compartments separated and net fresh weights determined.

Relation of empty stomach to weight of the fetus was computed as a percentage. Weights of the rumen, reticulum, omasum and abomasum were compared as percentages of the empty stomach. Some observations were made as to stage of development of the separate compartments.

Of 34 individuals used in this study, 29 were single-birth fetuses obtained by slaughter. A cow due to calve soon was slaughtered to secure records of the placenta, fluids and fetus at full term, thus providing the 278-day fetus. Triplet females were born prematurely and dead at 250 days in gestation. Records of the latter are presented, but were not considered in any deductions. Two male calves were born at 268 and 283 days, but suffocated at birth. All were from registered Jerseys, except male 113-day and 118-day Guernsey fetuses and a female 228-day Jersey-Guernsey fetus.

RESULTS

Early in the gestation period, the empty stomach comprised over 1.8 per cent of the total fetus by weight, decreasing in proportion from about the sixth month. It amounted to approximately 1.3 per cent of total body weight at full term, as seen in table 1.

A 59-day male fetus was the youngest individual used for dissection. The separate stomach compartments appeared from the external surface to be differentiated, but were too small for gross dissection. The reticulum from a 72-day fetus was insufficiently differentiated for a satisfactory gross separation from the rumen.

With older fetuses up to 4 mo. from date of service, the rumen dominated

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in net weight, representing two to three times the weight of the abomasum. However, after about 4 mo., the abomasum began to increase more rapidly than other compartments. The abomasum and rumen were about equal in weight at 6 mo. At full term, the abomasum weighed about as much as the other three compartments combined. Such development of the abomasum may be related to the fact that it must function at birth. Other compartments probably require further postnatal development before being adapted for full function.

TABLE 1

Development of the bovine stomach during fetal life

Age	Sex	Fetus wt.	Stomach and contents	Empty wt.					Relation of empty stomach to fetus weight
				Rumen	Reticulum	Omasum	Abomasum	Total	
(*d.*)			(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(%)
59	M	11.94 g.	0.28[a]	.....	.....	.....	.....	.....	.....
72	M	43.24	0.88	0.19	0.09	0.164	0.091	0.535	1.24
74	F	44.00	1.80	0.28	0.08	0.23	0.14	0.73	1.66
77	M	56.66	2.38	.....	not dissected		.....	1.24	2.19
81	M	89.00	.....	0.67	0.15	0.44	0.39	1.65	1.85
89	M	172.5	6.03	1.38	0.24	0.82	0.42	2.86	1.66
93	M	227.00	.....	1.69	0.40	0.99	0.72	3.80	1.67
100	F	284.00	23.14	2.73	0.62	1.95	0.83	6.12	2.16
109	M	1.08 lb.	27.67	4.28	0.65	2.78	1.45	9.16	1.87
113	M	1.07	18.22	4.21	0.75	2.70	1.41	9.07	1.87
118	M	1.10	14.60	4.16	0.64	2.82	1.38	9.00	1.80
121	M	2.90	33.75	6.91	1.57	4.90	3.86	17.24	1.31
127	F	1.96	20.00	7.12	1.64	4.12	3.47	16.35	1.84
127	M	2.18	64.48	9.00	1.55	4.31	4.84	19.70	1.99
138	M	3.70	.....	11.51	2.37	8.07	6.40	28.35	1.69
148	F	4.0	.....	12.62	3.86	8.80	8.34	33.62	1.85
151	F	4.4	67.0	13.52	2.93	12.07	7.85	36.37	1.82
155	F	6.2	113.00	18.10	4.73	14.73	12.99	50.55	1.80
156	M	8.9	260.7	25.76	6.69	23.07	16.71	72.33	1.79
177	F	10.1	279.00	27.46	5.32	16.09	25.67	74.54	1.63
186	M	15.0	282.00	31.88	8.38	26.82	37.03	104.11	1.53
204	F	19.1	359.00	43.13	12.03	33.70	46.77	135.63	1.57
207	M	21.5	486.3	46.9	10.3	34.7	59.5	151.4	1.55
228	F	30.5	.....	73.56	16.69	34.10	87.33	211.68	1.53
231	M	36.0	450.00	75.00	19.00	42.00	92.00	228.00	1.48
235	M	28.00	.....	58.00	13.5	31.00	83.5	186.00	1.46
236	F	42.00	528.00	70.75	20.19	40.20	85.23	216.37	1.14
245	M	41.00	258.00	78.90	15.52	50.04	93.35	237.81	1.28
250 triplets premature	F	30.00	.....	72.00	16.00	20.00	76.00	184.00	1.35
	F	29.5	.....	66.00	14.00	22.00	66.00	168.00	1.26
	F	25.5	.....	66.00	14.00	16.00	56.00	152.00	1.31
268	M	58.00	.....	132.00	28.00	52.00	151.00	363.00	1.38
278	M	61.45	828.00	100.00	22.73	50.99	183.00	356.72	1.28
283	M	50.00	.....	100.00	24.00	36.00	145.00	305.00	1.34

[a] Stomach, intestines and mesentery combined.

No consistent trend was noted between weights of the rumen and reticulum at the different ages during fetal life. Some variations in weights may have been occasioned by the difficulty of making an accurate separation between the first and second compartments because of small size of the organ.

The rumen constituted approximately 30 to 36 per cent of total stomach weight from the sixth month of fetal life. It weighed four times as much as the reticulum from the eighth month on to full term.

During early fetal life the omasum ranked second to the rumen in weight, but generally was heavier than the abomasum up to 5.5 mo. At 7.5 mo., however, the omasum was a less prominent part of the total stomach. The rumen had attained double the weight of the omasum at time of birth.

The abomasum, intended by nature to function first, was the heaviest organ at birth, and the rumen ranked second. The reticulum was consistently the lightest in weight of the compartments.

Histological examinations were not made. The inner membrane of the abomasum appeared turgid and ready to function in fetuses dissected at full term. Musculature of the rumen wall, and the papillae which line it, were not developed strongly at birth. The honeycomb structure of the reticulum stood out distinctly as early as 3.5 mo. Generally, the omasum appeared incompletely developed at full term. The laminae inside the omasum developed rapidly between the 72nd and 100th days. They appeared flaccid, and the papillae on their surface and median borders lacked prominence even at birth.

SUMMARY

Development of the bovine stomach during fetal life is presented, based upon observations of 30 fetuses and two full-term calves obtained upon slaughter or at birth from dairy cows.

In early fetal life the rumen was largest of the four stomach compartments. At full term, however, the abomasum weighed about one-half as much as the total stomach. Differentiation of the honeycomb in the reticulum was noticed between the 72nd and 100th days. Papillae on laminae in the omasum developed slowly. The empty stomach comprised approximately 1.8 per cent of total fetal weight during early development, decreasing noticeably from 6 mo., to about 1.3 per cent at full term.

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SOME OBSERVATIONS ON THE HIGH-TEMPERATURE SHORT-TIME PASTEURIZATION OF CHOCOLATE MILK[1]

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The increased use of high-temperature short-time (HTST) pasteurization of whole milk has resulted in a desire by industry to employ this process for other milk products. In plants having a HTST unit it usually would be very desirable from the standpoint of operational efficiency to pasteurize chocolate milk also by this method.

Before adopting a process commercially, however, it should be examined with regard to its safety from the public health standpoint. The Milk Ordinance and Code recommended by the U. S. Public Health Service (4) stipulates that products such as chocolate milk be given minimum pasteurization treatments of 143° F. for 30 min. or 160° F. for 15 sec. The same times and temperatures are stipulated for the pasteurization of whole milk, which assumes that the thermal resistance of bacteria is of the same order in both whole and chocolate milk.

Although temperatures above those recommended by the Code normally are used in industry, it is desirable that data be available regarding the minimum times and temperatures required to adequately pasteurize chocolate milk.

EXPERIMENTAL

Laboratory studies. In line with the popular custom of manufacturing chocolate milk of the non-settling type, the present investigation concerned this type of product only. Formulae for the products were based on the results of a study of the optimum composition of chocolate milk conducted by Roberts *et al.* (2). Combinations of the different concentrations of the following ingredients used were: Kraystay (stabilizer)—0.0555 and 0.07 per cent (to give viscosities of 7.95 and 19.87 centipoise at 20° C.); sugar—5 and 8 per cent; non-fat dry milk solids—0 and 3 per cent; cocoa—1 per cent; whole milk to give 3.25 per cent butterfat in the final mix.

The laboratory phase of the study was concerned with the measurement of the heat treatments required to effect 99.99 per cent destruction of *Micrococcus freudenreichii* (no. MS66) in the various chocolate milk mixes and whole milk. This organism had been reported (3) to possess the heat resistance of *Escherichia coli* (no. 3U). The latter organism has been used extensively as a test organism in pasteurization and sterilization studies because its heat resistance was greater than the most heat-resistant pathogen. The technique for handling the test culture and carrying out the pasteurization studies was that reported by Speck (3) in a similar study on the HTST pasteurization of ice cream mixes.

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The holding times used at the different temperatures were as follows: 143° F.—35, 40, 45, 50, 55 and 60 min.; 145° F.—10, 15, 17.5, 20, 25 and 30 min.; 150° F.—1, 3, 5 and 7.5 min.; 155° F.—0.5, 1, 2 and 3 min.; 160° F.—5, 10, 12.5, 15, 17.5 and 20 sec.; and 165° F.—1, 2, 3, 4 and 5 sec. Tubes of milk similarly inocluated, but unheated, served as controls for the numbers of bacteria present prior to pasteurization. Usually two to four complete experiments were conducted at each temperature on each mix.

One ml. of the pasteurized material and suitable dilutions of the controls were plated on desoxycholate lactose agar (BBL). After incubation at 37° C. for 3 days, the pink and red colonies developing from surviving cells of the test culture were counted. Under these conditions colony formation by the micrococcus was not inhibited, and contaminants could readily be detected.

Plant studies. This phase of the work was conducted in the College creamery using a commercial plate pasteurizer, and the reduction of the total bacterial count was used as the index of pasteurization efficiency. The chocolate milk mix was prepared from a commercial non-settling cocoa preparation, or from 0.9 per cent American process cocoa with sufficient stabilizer added to give a viscosity of about 8 centipoise (at 20° C.). The mix also contained 5.0 per cent cane sugar and the butterfat content was standardized to 2.5 per cent by combining skim-milk and cream or by adding reconstituted non-fat dry milk solids to whole milk. The blending of these raw ingredients was accomplished by first mixing the stabilizer and cocoa, or the non-settling cocoa preparation, with a volume of hot milk (160° F.) equivalent to about 8 per cent of the final volume of the mix. This was stirred thoroughly for 5 to 10 min. It then was mixed with the remainder of the cold milk, to which the sugar had been added, and blended in a vat equipped with a stirrer. The complete mix was agitated continuously until the batch was pasteurized. This method was found essential in order to obtain uniform distribution of the stabilizer and also to provide for its proper dispersion during pasteurization.

A sample of the raw mix was collected from each batch, part of which was used as a control for counts on the unpasteurized product. Also, two 5-ml. portions were pasteurized in the laboratory, one at 160° F. for 20 min. and the other at 145° F. for 30 min. These latter two were used as pasteurized controls for evaluating the efficiency of the plant HTST pasteurization treatments. The first was used because it is the customary one for manufacturing non-settling chocolate milk, the second because it appeared, after completing the laboratory studies, to be the minimum low-temperature procedure that can be used with safety.

The raw mix was pasteurized in the HTST unit, starting with the highest pasteurization temperature under test and dropping to the succeeding lower temperatures. Usually, observations on temperatures of 175, 168 and 161° F. for holding times of 19 and 40 sec. were made for each batch. To attain the 40-sec. holding time, the heated milk was routed through an extra length of holding tube. Timing measurements were made on each experiment with a Foxboro Dynalog Electronic Timer. At each pasteurization temperature, ample time was allowed for the flushing of the pasteurizer with the product being pasteurized before

TABLE 1

Observed average times (with standard errors) at different temperatures to accomplish 99.99% kill of M. freudenreichii (no. MS66) in chocolate milk of various compositions

Mixture	Added non-fat solids[a]	Temperature—(° F.)					
		143	145	150	155	160	165
	(%)				(*min.*)		
Whole milk	0.0	32 ± 9	18 ± 3	4.3 ± 1.0	0.48 ± 0.14	0.15 ± 0.04	0.042 ± 0.009
Choc. milk (0.0555% Kraystay, 5% sugar)	5.8	44 ± 9	20 ± 3	3.6 ± 1.0	0.65 ± 0.19	0.18 ± 0.04	0.023 ± 0.009
Choc. milk (0.07% Kraystay, 5% sugar)	5.8	43 ± 9	21 ± 3	5.3 ± 0.8	0.76 ± 0.11	0.16 ± 0.04	0.046 ± 0.007
Choc. milk (0.0555% Kraystay, 8% sugar)	8.8	51 ± 9	21 ± 3	5.0 ± 1.0	0.59 ± 0.11	0.19 ± 0.03	0.053 ± 0.006
Choc. milk (0.07% Kraystay, 8% sugar)	8.8	48 ± 9	18 ± 6	5.2 ± 1.0	0.98 ± 0.14	0.19 ± 0.04	0.058 ± 0.009
Choc. milk (0.0555% Kraystay, 5% sugar, 3% NFDMS)	8.8	50 ± 7	24 ± 6	6.3 ± 1.0	0.86 ± 0.14	0.18 ± 0.04	0.058 ± 0.009

[a] Includes sugar, Kraystay, the solids-not-fat in the cocoa and NFDMS (non-fat dry milk solids).

collecting a sample. Samples of the finished product were collected from the line leading out of the cooling section of the HTST unit, through an outlet valve which had been thoroughly steamed.

Samples were plated on tryptone-glucose-extract skimmilk agar (Difco) for the total counts and plates were incubated at 35° C. for 48 hr. For coliform counts, the samples were plated on desoxycholate lactose agar (BBL) and the plates incubated at 35° C. for 18 to 20 hr.

RESULTS

The data were analyzed and summarized using statistical procedures outlined in the appendix. The results obtained are given in the following paragraphs.

Laboratory studies. The average times required to yield 99.99 per cent kill of the test organism ("critical" times) in the several mixtures at the several temperatures are shown in table 1, along with their standard errors. The mean values show some irregularity in trends related to temperature and solids-not-fat contents, but, as indicated by the standard errors, the irregularity is reasonably attributed to experimental error.

The data in table 1 were found to be very closely fitted by the equation

$$\log t_s = 21.103 - 0.137\ T + 0.014\ S \qquad \text{(Eq. 1)}$$

where t_s = the time required in minutes to give 99.99 per cent kill, T = pasteurization temperature (° F.) and S = solids-not-fat added to the milk in per cent (*i.e.*, solids-not-fat above natural milk content). Values corresponding to those in table 1, as obtained by equation 1 along with their standard errors as obtained from equation 2, are shown in table 2.

TABLE 2

Predicted times (with standard errors) at different temperatures to accomplish 99.99% kill of M. freudenreichii (no. MS66) in milk containing various amounts of added non-fat solids

Added non-fat solids[a]	Temperature—(° F.)					
	143	145	150	155	160	165
(%)				(*min.*)		
0.0	33 ± 5	17 ± 2	3.6 ± 0.4	0.74 ± 0.07	0.15 ± 0.02	0.031 ± 0.005
5.8	39 ± 5	21 ± 3	4.3 ± 0.4	0.89 ± 0.08	0.18 ± 0.02	0.038 ± 0.006
8.8	43 ± 6	23 ± 3	4.7 ± 0.5	0.98 ± 0.10	0.20 ± 0.02	0.042 ± 0.007

[a] Includes sugar, Kraystay, solids-not-fat in the cocoa, and NFDMS. Levels of solids selected to correspond with those in table 1.

The coefficients of T and S were both statistically significant. This reflects the well known fact that increasing the temperature decreases the required pasteurization time, but also shows that increasing the solids-not-fat content of the mix *increases* the required pasteurization time. Statistical tests on the effects of sugar, stabilizer, chocolate and milk solids were made. No evidence was obtained that these factors affected results in any way other than through the solids they contributed.

The variance from the true plane of any point estimated by equation 1 is estimated by

$V\ (\log\ t_s) = 0.001116 + 0.000018(T - 153)^2 + 0.000014(S - 4.9)^2$ (Eq. 2). If one uses equation 1 to predict the "critical" time for any single batch of milk, a quantity, 0.008932, must be added to the value given by equation 2, in order to estimate the prediction error variance.

Plant studies. The plant results are shown in table 3. The average plate counts for the several plant treatments are expressed as percentages of those obtained by the two laboratory check procedures. The standard errors of these percentages also are given. So that the reader may have an idea of the actual plate counts involved, it should be noted that the average plate count on laboratory method II was 356 ± 22 colonies.

TABLE 3

The destruction of the natural flora in chocolate milk by plant pasteurization as compared with laboratory controls

Treatment	Temperature	Time	Standard plate counts	
			As % of laboratory I	As % of laboratory II
	(° *F.*)	(*min.*)		
Lab. I	160	20	100	74 ± 9
Lab. II	145	30	134 ± 16	100
		(*sec.*)		
Plant 1	175	19	115 ± 14	85 ± 8
" 2	175	40	114 ± 17	85 ± 10
" 3	168	19	134 ± 15	100 ± 11
" 4	168	40	118 ± 16	88 ± 10
" 5	161	19	136 ± 17	101 ± 10
" 6	161	40	133 ± 17	99 ± 11

DISCUSSION

Laboratory studies. Because of the "smoothing" of the data which occurs due to using equation 1, the figures in table 2 are more precise than those in table 1. Hence, those in table 2 will be referred to in this discussion. Present observations on the heat resistance of this culture in whole milk agree quite well with those made in an earlier study (3). The differences existing appear to be attributable to experimental errors and to a somewhat less efficient analysis of the earlier data.

The addition of solids-not-fat which occurs in the making of chocolate milk substantially increases the time required for pasteurization. In the case of the mixtures having the lower solids content the increase was about 20 per cent at all temperatures, and for the mixtures having the higher solids content, about 30 per cent. This large relative increase is probably of no practical importance at the high temperatures. For example, at 165° F. it amounts to less than 1 sec. of holding time and at 160° F. it amounts to 3 sec. or less. On the other hand, the increase is of practical importance at the lower temperatures, since, for the high-solids mixtures, the increase was 10 min. at 143° F., and 6 min. at 145° F.

In comparing the data of table 2 with the specifications of the Milk Ordinance and Code, it appears that the recommended holding time of 30 min. at 143° F. is not adequate for the pasteurization of chocolate milks. Rather, a time of 49 min.

appears necessary. Raising the recommended temperatures to 145° F. would, however, allow the use of 30 min. as the holding time.

Although destruction of the test organism in chocolate milk at 160° F. was completed within 15 sec. holding time recommended by the Code, the margin of safety is not as great as that obtained with whole milk pasteurized at the same temperature for the same time. Since no deleterious effects on the product are encountered at 165° F., raising the recommended temperature to 165° F. for a holding period of 15 sec. would provide an adequate margin of safety. Although 15 sec. at 165° F. is not sufficiently long to produce a satisfactorily stabilized chocolate milk, as will be brought out later, it nevertheless is adequate from the public health standpoint.

Plant studies. It is seen from table 3 that all of the plant procedures, *i.e.*, 161, 168 and 175° F., each at holding times of 19 and 40 sec., gave destructions of organisms of the same order as did laboratory method II (145° F. for 30 min.). In fact, 175° F. for both 19 and 40 sec., and 168° F. for 40 sec. appeared to have somewhat greater bactericidal action than did 145° F. for 30 min. These three treatments thus are adequate from the public health standpoint, although they did not give as low bacterial counts as did laboratory method I (160° F. for 20 min.), which is the customary commercial procedure used to attain a non-settling product.

Aside from the bacteriological considerations in the use of 175° F. for 19 or 40 sec. and 168° F. for 40 sec., it should be noted that these treatments produced products in which the chocolate was satisfactorily stabilized. In contrast, pasteurization at 168° F. for 19 sec. and at 161° F. for 19 or 40 sec. usually gave products in which the chocolate was not completely stabilized. It thus appears that, under the usual practical conditions, the holding times required to provide adequate stabilization at high temperatures (160 to 175° F.) also will provide adequate pasteurization.

Temperatures higher than 175° F. were not studied in detail, since 180° F. caused a slight burnt-chocolate flavor.

SUMMARY

The present study indicates that the present standard of 160° F. for 15 sec. for the HTST pasteurization of chocolate milk will yield a product which is safe from the public health standpoint, although the margin of safety is not as great as that obtained with whole milk. A temperature of 165° F. for 15 sec., appeared to be advisable. The minimum standard of 143° F. for 30 min. for the holder method was not adequate for the destruction of the test organism *M. freudenreichii* (no. MS66), indicating that longer holding time or a higher temperature should be used to insure the safety of chocolate-milk. Here, 143° F. for 49 min. or 145° F. for 26 min. appeared adequate.

HTST pasteurization of chocolate milk at 161° F. for 19 or 40 sec. and at 168° F. for 19 sec., using a commercial plate pasteurizer, gave a reduction in the total count comparable to laboratory pasteurization at 145° F. for 30 min., but did not usually yield a well-stabilized product. HTST pasteurization at 175° F.

for 19 and 40 sec. and at 168° F. for 40 sec. gave bacterial destruction somewhat greater than that obtained at 145° F. for 30 min., but less than that obtained by laboratory pasteurization at 160° F. for 20 min. Well-stabilized products were obtained in these cases.

An equation is presented for predicting the minimum pasteurization time required for whole milk and for chocolate milk of various non-fat-solids contents over a range of pasteurization temperatures from 143 to 165° F. or slightly above.

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Statistical Appendix

The following is given to outline the general approach used and to point out some of the problems met in analyzing the data. Details on the computational procedures may be obtained by contacting the junior author.

Laboratory studies. According to theory and experiment (Rahn, 1932), the survival of bacteria subjected to high temperatures is closely approximated by the monomolecular law,

$$n_t = n_0 e^{bt} \qquad \text{(Eq. 3)}$$

where n_t = the number of bacteria living at time t, n_0 = the number of bacteria present initially ($t = 0$), e = the base of natural logarithms, b = the survival rate constant for a given organism at a given temperature in a given medium and t = time subjected to heat treatment. If one lets p_t be the percent surviving at time t, then equation 3 may be written as

$$p_t = 100e^{bt} \qquad \text{(Eq. 4)}$$

For statistical purposes equation 4 may be put in the form

$$\log\ p_t = \log 100 + bt \qquad \text{(Eq. 5)}$$

Theoretically, one needs to fit only the constant, b. Under the experimental conditions, however, there was obviously some error in the measurement of time, especially at high temperatures. It is proper, then, to write equation 5 in the form

$$\log\ p_t = \log 100 + b\ (t + d) \qquad \text{(Eq. 6)}$$

where d is the error in the measurement of time. It appeared safe to assume that d is predominantly a bias related to temperature, and has a negligible random component. This bias can then be taken into account by rewriting equation 6 as

$$\log p_t = a + bt \qquad \text{(Eq. 7)}$$

Thus, two constants, a and b, had to be fitted statistically. As would be expected, the deviation of a from log 100 was negligible at the lower temperatures, but was quite large at high temperatures. Hence, the fitting of a was justified.

Once a and b were obtained, the time required to allow a survival of only p_s per cent of the organisms was found as

$$t_s = \frac{\log p_s - a}{b} \qquad \text{(Eq. 8)}$$

The quantity t_s hereafter will be referred to as the "critical" time.

Equation 7 was fitted for each experiment on each mix at each temperature by standard regression procedures. These procedures were justified because the successive observations were independent, each having been made on a separate tube, and because preliminary tests showed the variance of $log\ p_t$ to be quite uniform along the regression line. The critical times then were determined for each experiment, using equation 8. For this purpose the per cent survival, p_s, was set at 0.01 per cent. The critical times so obtained were treated as the experimental observations in the remainder of the analysis.

The first computations on the critical times were those of obtaining the average values for each milk mixture at each temperature, and their standard errors (see table 1). The distribution of the critical times made geometric rather than arithmetic means the better to present. These were obtained, of course, by first taking logarithms. Standard computational procedures were carried out on the logarithms to arrive at the standard errors.

A preliminary plotting of the data showed that the logarithms of the critical times were linearly related to temperature. It also was found that the logarithms of the critical times were linearly related to the non-fat-solids contents of the milk mixtures. Statistical tests showed further that, over the ranges of temperature and solids contents involved, the effects of the two factors on the logarithms of the critical times were essentially additive.
Hence, the relation

$$\log t_s = b_0 + b_1 T + b_2 S \qquad \text{(Eq. 9)}$$

was fitted to the data (see equation 1 in the text). In equation 9 the b's are statistically determined constants, T is the pasteurization temperature in degrees of Fahrenheit, S is the percentage of solids-not-fat added to the milk in making any given mixture. The logarithms of the critical times, t_s, were taken to the base 10.

In fitting equation 9 it was found that the variances of $log\ t_s$ were not quite uniform along the regression plane, and, also, that the values for the several experiments at a given temperature were somewhat correlated. Various methods of fitting were tried. These included ordinary regression methods, which assume independence and uniform variance, as well as several methods involving weighting and taking into account the correlations. All gave values for the regression coefficients which were identical for all practical purposes.

The lack of independence and lack of uniform variance had to be taken into account, however, in arriving at the variance of "predicted" critical times (see equation 2 in the text). Expressed as variance about the true response plane which equation 9 estimates, the variance of a predicted value is

$$\begin{aligned} Var(\log t_s) = {} & Var\ (\log t_s) + (T-\bar{T})^2\ Var\ (b_1) \\ & + 2(T-\bar{T})\ (S-\bar{S})\ Cov(b_1, b_2) + (S-\bar{S})^2\ Var(b_2) \end{aligned} \qquad \text{Eq. 10)}$$

$Var(b_1)$ was estimated from the deviations of the temperature means from the regression plane. $Var(b_2)$ and $Cov(b_1, b_2)$ were estimated by pooling the deviations of the mixture means from the regression plane and variations within temperature-mixture subclasses. Despite the fact that the number of experiments on the several temperature-mixture combinations was not uniform, $Cov(b_1, b_2)$

was so small that it could be neglected. $Var(\log t_s)$ was estimated as an appropriate combination of the two basic components associated with $Var(b_1)$ and $Var(b_2)$. As is noted in the text, one needs to add another component to $Var(\log t_s)$ as given by equation 10, if it is desired to predict the critical time for a single batch of milk. This quantity also was obtained from the two basic components associated with $Var(b_1)$ and $Var(b_2)$.

Plant studies. These involved six different pasteurization treatments, all of the same milk mixture, which were carried out over a period of several months. Fourteen different batches of mixture were treated. On each mixture, one or both laboratory checks were made, but not all plant treatments were applied to each. On some batches only two of the plant procedures were applied, but as many as five were applied in several cases. The several batches differed considerably in initial count and type of bacteria, and this variability had to be removed from error to make precise comparisons. The data were thus nonorthogonal and it was necessary to use basic least squares for the analysis.

The analysis was conducted on the logarithms of the plate counts. This rendered the variance essentially homogeneous, and, in addition, made for easy comparison of the plate counts of the plant treatments as percentages of the plate counts obtained with the laboratory checks (table 3).

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TOXICITY TO BULL SPERMATOZOA OF VARIOUS SALTS, BRANDS AND LOTS OF PENICILLIN, STREPTOMYCIN, AUREOMYCIN AND CHLOROMYCETIN[1]

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The control of bacterial growth in diluted bull semen to be used for artificial insemination is a matter of major concern, since such control may result in increased fertility levels in some bulls. Considerations relative to the choice of antibiotics, manufacturers' brands, antibiotic salt or complex and dosages to be employed are of great importance especially in regard to semen toxicity, a major criterion of the possible usefulness of a new material.

Foote and Salisbury (6), in 1948, reported that one of two manufacturers' brands of penicillin G was toxic to bull spermatozoa even in small amounts, emphasizing the need for a current evaluation of progress in the manufacturing field. Almquist *et al.* (1, 2, 3) have reported on the semen toxicity of sodium penicillin G (Pfizer) and on streptomycin sulfate (Pfizer) each alone and in combination, but information is lacking on the potassium salt of penicillin G or on other salts of streptomycin, including dihydrostreptomycin. Welch *et al.* (12) showed that when penicillin G is combined with various cations, the cation contributes substantially to the toxicity of the preparation as measured in mice. Reporting in 1944, Welch *et al.* (13) found wide variation in the acute toxicity to mice of different manufacturers' brands of penicillin.

Molitor (10) reported in 1946 that the toxicity of a streptomycin in various animals was greatly affected by traces of impurities present.

Myers *et al.* (9), working with aureomycin hydrochloride, reported that all levels studied had significant toxic effects on bull spermatozoa motility. Foote and Bratton (7), also working with aureomycin hydrochloride in bull semen, reported that to avoid spermicidal effects the dosage should not exceed 100γ per milliliter of diluted semen.

Chloromycetin (Parke-Davis) is an antibiotic which is similar to aureomycin in its effectiveness against a wide range of gram positive and negative organisms and against some of the large viruses (4, 8, 14).

METHODS AND MATERIALS

Two lots each of four manufacturers' brands of penicillin G (including both the Na and K salts) were studied as to their relative semen toxicity. Similarly, studies on two lots each of three brands of streptomycin (including the sulfate, hydrochloride and calcium chloride complexes) were made. One lot of dihydrostreptomycin sulfate, both the base and hydrochloride of aureomycin and chloromycetin also were studied as to their semen toxicity reactions (table 1).

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Six dairy bull semen samples were used in each toxicity study and the semen was diluted 1:20 with an egg yolk-citrate diluter consisting of 1 part egg yolk and 3 parts of 3 per cent sodium citrate dihydrate to which the antibiotic was added. One-ml. portions of diluted semen were stored at approximately 5° C. in 1.5-ml. stoppered tubes. The diluted semen samples were examined on storage

TABLE 1

Brands, salts and lot numbers of antibiotics used in study

Antibiotic	Brand	Salt	Manufacturers' lots	
			1	2
Penicillin G	Lilly	Potassium	7317–467340	9317–471437
	Merck	Sodium	275	295
	Pfizer	Sodium	668	678
	Squibb	Sodium	17143	17147
Streptomycin	Merck	$CaCl_2$ Complex	1599	1903
	Pfizer	Sulfate	48179	48209
	Squibb	Hydrochloride	11875	11876
Dihydro-streptomycin	Lilly	Sulfate	8318–493979	
Aureomycin	Lederle	Base and		
Chloromycetin	Parke-Davis	Hydrochloride		

days 5, 10 and 15 and the percentages of motile spermatozoa estimated. The data were analyzed by the analysis of variance method (11).

RESULTS

Penicillin. A summary of the spermatozoa motility data on penicillin is presented in table 2. These data are presented in an abbreviated form since an

TABLE 2

Spermatozoa motility studies with penicillin G

Day	5	10	15
Dosage	Motile spermatozoa (%)		
(*mg./ml.*)			
0	41.1	14.8	5.7
500	43.2	16.4	7.8
1000	43.6	16.5	9.0
2000	42.4	16.3	8.6
4000	39.7	15.5	6.4
8000	27.2	4.8	0.3

analysis of the original data indicated that no significant differences existed between the contrasted means for brands, salts or lots of penicillin. The dosage effects were highly significant, and thus, 4,000 units of penicillin per milliliter of diluted semen may be considered a non-toxic level, while the 8,000 unit level is only mildly toxic.

Streptomycin. No significant differences in spermatozoa motility could be attributed to the brands, salts or lots of streptomycin used. Accordingly, only dosage and time effects are presented in the data summary of table 3. A highly

TABLE 3

Spermatozoa motility studies with streptomycin

Day	5	10	15
Dosage	Motile spermatozoa (%)		
(*mg./ml.*)			
0.0	45.4	18.3	6.1
0.5	46.9	24.8	8.5
1.0	47.3	25.3	7.5
2.0	46.1	24.9	9.0
4.0	44.4	21.9	8.3
8.0	42.9	19.4	5.9

significant dosage effect was found, and an examination of the mean motility data indicated that a beneficial effect was exerted on motility characteristics of the stored semen by certain streptomycin dosage levels. The observation of Easterbrooks *et al.* (5) that the $CaCl_2$ complex of streptomycin forms a precipitate on dilution and storing at 5° C. was confirmed.

Dihydrostreptomycin sulfate. One lot of dihydrostreptomycin sulfate was studied as to its semen toxicity properties (table 4). Analysis of variance showed

TABLE 4

Spermatozoa motility studies with dihydrostreptomycin sulfate

Day	5	10	15
Dosage	Motile spermatozoa (%)		
(*mg./ml.*)			
0.0	43.3	24.2	14.0
0.5	45.0	29.5	14.8
1.0	42.5	29.0	13.5
2.0	43.3	29.8	14.3
4.0	41.7	29.2	10.7
8.0	40.8	27.5	14.2

no dosage effect upon the percentage of motility, and dosages as high as 8.0 mg. per milliliter of diluted semen are judged to be non-toxic, being similar to streptomycin in this respect.

Aureomycin. Comparison of aureomycin base and the hydrochloride showed no differential effects on semen motility. The initial toxic level was found to be 0.125 mg. per milliliter of either the base or hydrochloride, the 0.0625-mg. level being non-toxic (table 5).

TABLE 5

Spermatozoa motility studies with aureomycin

Day	5	10	15
Dosage	Motile spermatozoa (%)		
(*mg./ml.*)			
0.0	51.9	32.5	16.9
0.0625	52.8	34.1	15.0
0.125	50.0	25.6	8.9
0.25	45.9	21.6	5.8
0.50	35.0	10.2	0.9
1.00	19.4	4.6	0.2

Chloromycetin. One lot of chloromycetin was available for study. Analysis of the data (table 6) showed highly significant dosage effects, which were most

TABLE 6

Spermatozoa motility studies with chloromycetin

Day	5	10	15
Dosage	Motile spermatozoa (%)		
(*mg./ml.*)			
0.0	50.0	30.8	13.3
0.125	53.3	35.8	10.8
0.25	52.5	30.8	12.0
0.5	51.7	33.3	9.7
1.0	51.7	29.2	7.8
2.0	46.7	21.7	3.8

pronounced at 10 and 15 days' storage. The initial toxic level was judged to be 1.0 mg. per milliliter. On this basis, chloromycetin is somewhat less toxic to spermatozoa than aureomycin but more toxic than penicillin, streptomycin or dihydrostreptomycin.

DISCUSSION

This limited survey of manufacturers' brands, salts and lots of penicillin and streptomycin relative to semen toxicity seems to indicate a rather satisfactory general condition, both from the standpoint of purity (low toxicity) and of comparative toxicity of the various salts or complexes of these antibiotics. If direct comparison could be made with the earlier toxicity work of Almquist and coworkers with both penicillin and streptomycin (this may not be strictly valid) it would seem that initial toxic levels in both instances were raised considerably in the present study, indicating perhaps better manufacturing procedures in purification of the antibiotics. Differential toxicity due to the various salts or complexes of the antibiotics could not be demonstrated in this study.

The toxicity data for aureomycin agree well with those reported by Myers *et al.* (9) and Foote and Bratton (7).

The relatively low toxicity of chloromycetin seems to warrant further consideration of this antibiotic for inclusion in the antibiotic mixture of diluted semen.

SUMMARY

A limited survey was made of the toxicity to bull spermatozoa of several antibiotics including four brands and two salts of penicillin, three brands and three salts of streptomycin, one lot of dihydrostreptomycin, two salts of aureomycin and one lot of chloromycetin. Differences in spermatozoa motility were not attributed to brands, lots or salts of either penicillin or streptomycin. Dihydrostreptomycin sulfate was not toxic to spermatozoa at levels as high as 8 mg. per milliliter of diluted semen. The initial toxic level of aureomycin was found to be above 0.0625 mg. per milliliter while that of chloromycetin was found to be above 0.5 mg. per milliliter of diluted semen.

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THE EXTRACTION AND HYDROLYSIS OF THE ANDROGENS OF COW MANURE[1]

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Riley and Hammond (3) discovered that the feeding of dried cow manure to day-old chicks caused marked stimulation of comb growth. Evidence was presented indicating that the factor present was an androgenic rather than a gonadotrophic substance.

Turner (4, 5, 6) confirmed the report of the presence of orally-active androgens in the feces of dairy cows and heifers when dried at 45° C.

Some information is available as to the properties of these androgenic substances. Hammond (1) demonstrated that the fecal androgens were destroyed by high-temperature drying of the feces, and Turner (6) reported that in cow manure the activity was an inverse function of the drying temperature above 45° C.

Using 50 to 60 per cent ethanol, Riley and Hammond (3) reported that they obtained biologically active extracts of dried cow manure. Their work also indicated the solubility of fecal androgens in chloroform. Longwell and Gassner (2) have reported the solubility of fecal androgens in ethanol, presumably in the commercial concentration of 95 per cent, but reported the androgens only slightly soluble in chloroform.

The evolution of a successful method of purification and isolation of the androgenic substances in feces depends largely on the determination of the state in which the hormones exist, *i.e.*, free or bound, for their physical and chemical properties naturally depend on the state in which they may exist.

The present paper presents the results of a series of experiments in which cow manure dried at 45° C. was extracted with a number of solvents of various types. The results of these studies are believed to throw light on the nature of the state of conjugation of the hormones.

EXPERIMENTAL[2]

Extraction of dried cow manure with various solvents. The efficiency of various solvents for the extraction of the androgenic factors was determined. The solvents used were methyl, ethyl and butyl alcohols, acetone, petroleum ether, ethyl ether, benzene, toluene, carbon tetrachloride, chloroform, ethylene dichloride and water. The fresh manure was collected from individual cows of the Guernsey, Holstein and Jersey breeds in the University of Missouri dairy herd and dried according to a procedure previously described (Turner, 1947).

A 1250-g. portion of dried cow manure was extracted several times with

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TABLE 1

Extraction of dried cow manure with various solvents

Treatment	Male chicks				Female chicks				M & F / 2
	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt./ 100 g. body wt.	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt./ 100 g. body wt.	
		(*g.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	
Control	9	245.6	89.3	36.4	11	207.3	43.1	20.8	28.6
Standard[a]	42	251.6	252.0	100.2	36	234.3	278.8	118.9	109.5
Dried cow manure	22	190.0	263.1	131.7	14	167.9	139.3	82.0	106.8
Methyl alcohol extract	8	295.9	309.1	104.5	12	281.3	225.7	80.2	92.4
Ethyl alcohol extract	21	162.5	175.7	108.1	1	146.0	132.6	90.8	99.5
Butyl alcohol extract	14	182.1	169.5	93.1	2	183.5	114.9	62.6	77.8
Acetone extract	5	214.0	258.9	121.2	12	213.0	172.6	80.9	101.1
Chloroform extract	20	152.1	75.7	49.6	10	139.2	56.8	36.0	42.8
Petroleum ether extract	10	218.2	88.6	40.6	11	201.9	63.2	31.3	35.9
Ethyl ether extract	8	175.3	57.1	32.1	11	168.3	42.8	25.4	28.7
Benzene extract	11	178.8	71.7	39.9	9	177.6	58.2	32.8	36.4
Carbon tetrachloride extract	7	323.0	142.5	44.2	15	302.1	75.4	24.9	34.6
Toluene extract	13	224.8	88.4	39.3	6	201.2	51.4	25.6	32.5
Ethylene dichloride extract	8	301.3	121.8	40.4	10	291.8	69.5	23.8	32.1
Water extract	10	226.0	117.9	52.2	9	204.0	44.6	21.9	37.1

[a] 10 mg. methyl testosterone/kg. feed.

large volumes of each of the several solvents using a continuous extraction apparatus. The solvents were removed by distillation, and the extracts remaining were assayed biologically for androgenic activity by the feeding method described (7). The bioassay results after solvent extraction were compared with methyl testosterone (10 mg. per kg. feed) and to a sample of unextracted dried cow manure.

These data indicate that the most effective solvents are methyl, ethyl and butyl alcohols and acetone. The results obtained with petroleum ether, ethyl ether, benzene, toluene, carbon tetrachloride, chloroform, ethylene dichloride and water indicate that the latter solvents extract very little androgenic activity from the dried cow manure (table 1).

Since it is well known that the free androgens are soluble in ethyl ether, benzene, carbon tetrachloride, chloroform and ethylene dichloride, it would appear that the androgens present in dried cow manure are not in a free or unconjugated form. This being true, it was of interest to determine if these biologically active extracts could be hydrolyzed to their free form soluble in the above solvents.

Effect of acid hydrolysis of an active extract. A 1250-g. portion of dried cow manure was extracted several times with large volumes of acetone as before. The acetone extract was concentrated to 1 l., and 15 per cent by volume of concentrated HCl was added. The mixture was refluxed 15 min. and cooled. The acid then was neutralized by the addition of 5*N* NaOH. After placing in a separatory funnel, the water containing the NaCl formed on neutralization was drained off. The acetone was distilled to dryness and the residue extracted several times with ethyl ether with refluxing. The ether was removed by distillation, and 16 g. of residue were obtained which were assayed as before (table 2).

The apparent result of this hydrolysis was to free the androgenic substances from a chemically bound or conjugated state and to render them ether soluble—a property possessed by the free androgens. Solubility in ether is used as the criterion for the effect of the hydrolytic treatment. Of course there is an inevitable loss of some activity due to hydrolysis.

Effect of a solvent exchange technique on the androgenic substances. It was found that the bound androgenic substances in cow manure could be freed by a much milder treatment. By using a solvent exchange technique it also was possible to obtain an active ether extract. A 1250-g. portion of dried cow manure was extracted several times with large volumes of ethyl ether in a continuous extraction apparatus. The ether extract was discarded. The residue in the continuous extraction apparatus then was extracted with 95 per cent ethanol. The ethanol was removed by distillation and the extract stirred under reflux with two separate portions of 60 per cent ethanol. The combined 60 per cent alcohol extracts were distilled to dryness. This residue then was extracted repeatedly with ethyl ether and the ether removed by distillation. Twelve g. of residue remained which were bioassayed and found to be active (table 3). The final active ether extract indicates that the combined or adsorbed androgenic sub-

TABLE 2

Effect of acid hydrolysis of an active extract

Treatment	Male chicks				Female chicks				M & F / 2
	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt. /100 g. body wt.	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt. /100 g. body wt.	
		(*g.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	
Control (table 1)	9	245.6	89.3	36.4	11	207.3	43.1	20.8	28.6
Acetone extraction of cow manure (table 1)	5	214.0	258.9	121.2	12	213.0	172.6	80.9	101.1
Ether extraction of active fraction after acid hydrolysis	13	209.4	141.5	67.6	7	202.6	115.6	57.0	62.3

TABLE 3

Effect of a solvent exchange technique on the androgenic substances

Treatment	Male chicks				Female chicks				M & F / 2
	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt. /100 g. body wt.	No. of chicks	Av. body wt.	Av. comb wt.	Comb wt. /100 g. body wt.	
		(*g.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	
Control	9	245.6	89.3	36.4	11	207.3	43.1	20.8	28.6
Ether extraction of dried cow manure (table 1)	8	175.3	57.1	32.1	11	168.3	42.5	25.4	28.7
95% ethyl alcohol extract from ether extracted residue	5	248.0	323.0	130.3	5	205.2	290.2	141.4	135.6
Ether extraction of dried cow manure after solvent exchange	3	210.4	193.8	92.1	7	209.4	128.2	61.0	76.6

stances in cow manure could be released by this treatment also. Slightly better purification and activity was effected by this method than by the method of acid hydrolysis.

Comparison of assay by oral administration with comb application method. Additional information can be offered in support of the theory that the androgenic factors are not present in a free state in dried cow manure. An acetone extract of dried cow manure exhibited androgenic activity when fed orally to baby chicks. However, when this extract was bioassayed by the comb application method, no activity was noted. This indicated that the androgens after extraction from dried cow manure were in a bound or associated state and became active orally following hydrolysis or release in the digestive tract. Further verification of these results was obtained by using the acid-hydrolysis method and the solvent exchange technique on active extracts of dried cow manure. Assay of these extracts by the comb application technique indicated that these methods had liberated the androgens from a bound state for they were active when applied locally to the combs.

DISCUSSION

In the previous paper (8) it was shown that the male hormones in fresh cow manure are present in a form unavailable to chicks when given orally. Their availability to chicks was not increased by heat without desiccation. The question was raised whether desiccation made the hormones orally available by chemical hydrolysis of conjugated hormones. Drying of the fresh manure also could cause a molecular rearrangement in certain substances with the consequent production of orally active hormones. The possibility of the hormones being released from an adsorbed condition by the desiccation treatment also must be considered. An adsorbed condition of the hormones could be altered or decreased to the extent that dried cow manure exhibits oral androgenic activity in baby chicks, whereas fresh cow manure is inactive.

From the data presented in this paper concerning the most efficient solvents for extraction of the androgens from dried cow manure, it is evident that the desiccation had not completely released the hormones from a bound to a free state. The known free androgens are soluble in such solvents as ethyl ether, benzene, carbon tetrachloride, chloroform and ethylene dichloride. Since biologically active extracts were not obtained with these solvents, it was decided to attempt to hydrolyze the extracts in order to free the hormones and render them soluble in the above solvents. The ethyl ether extract contained considerable activity after acid hydrolysis of an active extract, as described in expt. 2. It was assumed, therefore, that the androgenic factor was present in the dried cow manure in a conjugated molecule and required vigorous hydrolysis in order to free it. This contention was also supported by the results obtained in expt. 1, since the solvents which were successful were solvents that would extract a chemically combined substance of this type.

However, when it was found that the mild treatment involved in the solvent exchange procedure could also effect ether solubility, it suggested that the hormones in the dried cow manure were not in a tight chemical combination but,

rather, were in a loosely associated or adsorbed state. The forces which cause adsorption may be either electrostatic or van der Waals forces which depend on the attraction of masses for one another. The solvent exchange procedure produced a marked decrease in total solids and, consequently, decreased the amount of surface area available for adsorption in the solvent extracts of these solid residues. Also, there is a certain degree of specificity in both forces that cause adsorption so that some substances are better adsorbents for certain adsorbates than are others. Therefore, the solvent exchange procedure, by the purification effected, also could decrease the amount of adsorption by eliminating the substances that are selective adsorbents for the androgenic hormones. These effects would enable the ether to extract the androgenic factors from a purified residue but not from the dried cow manure.

The decreased adsorption of the androgenic factors caused by acetone and alcohol extractions, as evidenced in the experimental results presented in this paper, indicates the specific ability of these solvents to decrease the amount of surface area and/or eliminate specific adsorbents of the hormones. It also is interesting to note the high dipole moment of these solvents and the possible relationship of the dipole moment of the solvent to an electrostatic adsorption of the hormone. It seems feasible to assume said electrostatic forces, if present, could be altered or the intensity of the electrical fields could be decreased by solvents possessing high dipole moments.

CONCLUSIONS

Data concerning the efficiency of extraction of dried cow manure with various solvents are presented. The solvents found to directly extract the androgenic factor from dried cow manure are methyl, ethyl and butyl alcohol and acetone.

Evidence is presented in support of the theory that the androgenic factor in dried cow manure is not in a free state but in a loosely associated or adsorbed form.

Two methods are presented for liberating or hydrolyzing the androgenic factors from their bindings.

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CRITERIA FOR JUDGING A TRUE RUMEN ORGANISM AND A DESCRIPTION OF FIVE RUMEN BACTERIA

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In recent years several investigators have isolated bacteria in pure culture from the rumen and have studied these bacteria. Kohler (8), Johnson *et al.* (7) and Van Der Wath (12) isolated bacteria from the rumen using aerobic technics. Pochon (9) isolated an anaerobic, spore-forming, cellulose-digesting rod from undiluted rumen contents, while Sijepsteijn (11) also has isolated several cellulose-digesting bacteria from raw rumen contents, but only a few were obtained in pure culture. Hungate (4, 5) isolated several anaerobic cellulose-digesting bacteria from diluted rumen contents, and analyses were carried out on the end-products formed when these organisms break down cellulose. Johns (6) used the Warburg technic to study the mechanism of propionic acid formation brought about by an anaerobic micrococcus obtained from the rumen of sheep.

Little work has been carried out to determine whether these organisms really are important rumen bacteria, capable of becoming established and functioning under rumen conditions or whether they are simply incidental bacteria found in the rumen. Also, no attempt has been made to determine how frequently these organisms occurred in ruminants. During studies in this laboratory, the question arose as to what criteria could be established to distinguish a true rumen organism from an incidental organism. A summary of the data obtained from a study of about 5,000 bacterial cultures isolated from the rumen during the past 5 yr. has been used as a basis for setting up the following five criteria for judging whether a bacterium isolated from the rumen is a true rumen organism. These criteria are: (a) anaerobiosis, (b) presence in numbers of at least one million per gram of fresh rumen contents, (c) at least ten isolations of a similar type bacterium from two or more animals, (d) isolation of similar type bacteria in at least two geographical locations and (e) production by the organism of end-products found in the rumen from substrates found in the rumen. This paper also contains a description of five rumen organisms that are typical of those used in collecting the data to set up these criteria.

EXPERIMENTAL TECHNICS

Samples of rumen contents from about 100 adult cattle and 100 calves in seven herds and from about 150 sheep in four flocks located in various parts of Ohio, New York and Tennessee were studied with respect to the kinds and numbers of bacteria present. About 5,000 isolations have been made and studied from these animals. The technics used included a direct slide count to determine the numbers of bacteria, Gram stains for observation of the original rumen flora and an anaerobic cultural series to obtain isolations of the predominating rumen

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organisms. Most of these samples were obtained by rumen tube, but a few were obtained from fistulated animals. The methods used for these experiments have been described in previous publications (2, 3).

The cultures isolated in field experiments were sent back to the central laboratory in Oakdale in agar shakes for further study. These cultures then were purified by the usual plating procedures, using anaerobic precautions. The agar was of the same composition as the broth used in the primary culturing technic described in a previous publication (2). After purification, the organisms were subjected to a series of "screen tests" for the purpose of comparing similar cultures, and to set up "type cultures" to determine the frequency of isolation of similar organisms. By the time the purification of the culture had been completed, the morphology and Gram reaction of the organism, type and rate of growth in the rich organic broth or agar, the final pH in broth and the degree of anaerobiosis were known. The physiology of these bacteria was studied by observing the reactions of the organisms in ten screen test media. A broth of composition similar to the medium used in primary culturing, with the beef extract omitted, was used, as well as seven similar broths in which the glucose was replaced by either fructose, maltose, sucrose, lactose, dextrin, starch or by no carbohydrate. The medium was autoclaved with the carbohydrate already in it, since broth prepared in this manner is easier to use, less liable to contamination and gives more clear-cut differences between the type cultures than the broth to which the sugar had been added aseptically after autoclaving. Since autoclaving some sugars in the presence of the rest of the medium alters the carbohydrates, the results of these tests must not be considered as fermentation reactions on the reactive carbohydrates. In addition, the reaction of the organism growing in litmus milk containing 1 per cent peptonized milk and the ability of the bacterium to proteolyze gelatin containing 1 per cent peptonized milk were tested.

In order to learn more about the physical characteristics of the type cultures, the bacteria were tested for their ability to form lactic acid or other organic acids from carbohydrates, to breakdown cellulose, and to synthesize the various B vitamins. Lactic acid was determined by a method described by Davidson (1), and the other organic acids such as propionic, butyric and acetic were determined by Craig's counter current distribution technic (10). Cellulose digestion was determined by analyzing for cellulose loss using the procedure described by Viles and Silverman (13). Microbiological assays for the B vitamins were carried out on a 3- to 5-day culture of the organism grown in the rich organic broth used for primary culturing. An increase or decrease in the amount of thiamine, riboflavin, pyridoxine, panthothenic acid, biotin, folic acid, niacin and B_{12} present in the medium was determined. Warburg tests were conducted on these organisms to determine the ability of the resting cells to act upon glucose, sucrose, cellobiose, urea, starch, lactic acid and glutamic acid. The stain used in testing for spore-formation was described by Wynne (14).

EXPERIMENTAL RESULTS

On the basis of the tests described above, about 20 type cultures have been established and their physiological characteristics and occurrence in the rumens

of cattle and sheep have been studied. The information obtained from the study of all these type cultures has been used to establish criteria for distinguishing true rumen organisms from incidental rumen organisms, but because of the limitations of space, the results from only five of the more common fast-growing organisms will be reported here, to illustrate the type of data used in setting up these criteria. These five organisms were chosen because their metabolism is quite similar and relatively simple and will, therefore, need little discussion. The other type cultures will be reported in a later paper where more space can be devoted to the discussion of their characteristics.

Table 1 shows the typical reactions of these five organisms when grown in the rich organic broth and agar used to isolate the primary cultures. Four of these five organisms were rods while one was a coccus. Most of the organisms were Gram positive when young, changing to Gram negative upon aging. All of these

TABLE 1

Characteristic reactions of 5 fast-growing rumen bacteria to the primary culture broth and agar

Designation of "type culture"	Morphology and gram reaction	Appearance and rate of growth in broth	Final pH in broth	End-products produced in broth	Anaerobiosis
RO-6TBR	Gram + cocci in pairs and short chains	Heavy turbidity in 10 hr., sediment	4.4–4.6	Lactic acid	Anaerobic
RO-L_1	Gram + irregular staining, pleomorphic, thick long rods	Moderate turbidity 16 hr., occasionally slight sediment	4.4–4.7	Lactic acid	Very strict anaerobe
RO-L_2	Gram + weak staining pleomorphic branching granulated rods	Heavy turbidity, 16 hr., sediment	4.3–4.6	Lactic acid	Very strict anaerobe
RO-L_3	Gram + short thin rods, often in raft formation	Moderate turbidity 16 hr., slight sediment	4.4–4.7	Lactic acid	Anaerobe
RO-T_1	Gram + tiny thin threadlike rod with Gram + granules	Slight turbidity, 16–24 hr., slight gas	4.4–4.8	Lactic acid and some gas	Very strict anaerobe

organisms were fast-growing bacteria which gave a recognizable turbidity in the broth and lowered the pH of the broth markedly. The lowering of the pH was due in all cases to the production of lactic acid. All organisms were anaerobes and none produced spores under the conditions of the tests.

Table 2 presents a summary of some of the physiological characteristics of these organisms. All of these organisms produced acid in almost all of the carbohydrate media. When filtered carbohydrates were added aseptically to the broth after autoclaving, the organisms produced acid from these carbohydrates, but at a slower rate. RO-L_1, RO-L_2 and RO-T_1 usually did not ferment the starch medium, and RO-T_1 produced gas whenever acid was produced. None of these organisms produced acid in the medium in the absence of carbohydrates. Growth in litmus milk usually produced an acid reduced curd and none of the organisms appeared to be proteolytic. The greatest differences in these organisms lay in their synthesis, or need for, some of the B vitamins; organisms RO-L_1, L_2 and L_3 are examples of this.

TABLE 2

Some physiological reactions of five rumen bacteria

Designation of "type culture"	Fermentation of broths containing									Litmus Milk	Vitamins	
	Starch	Dextrin	Maltose	Lactose	Sucrose	Glucose	Fructose	No-Sugar	Gelatin		Synthesized	Used
RO-6TBR	A[a]	A	A	A	A	A	A	O	O	ARC	Slight Biotin	O
RO-L_1	O	A	A	A	A	A	A	O	O	ARC	Folic Acid	Sl. PA
RO-L_2	Usually O	A	A	Usually A	A	A	A	O	O	Usually ARC	O	Thiamine, Riboflavin, PA & B_{12}
RO-L_3	Usually A	A	A	Usually A	A	A	A	O	O	ARC	B_{12} & PA	O
RO-T_1	O	AG	AG	AG	AG	AG	AG	O	O	ARC	O	PA, Biotin & B_{12}

[a] A—Acid
G—Gas
O—No acid or gas
ARC—Acid reduced curd
SL—Slight
PA—Pantothenic acid

Table 3 presents data on the number and distribution of the isolations of these five rumen bacteria. Photomicrographs of these five organisms are shown in figure 1.

CHARACTERISTICS OF BACTERIA

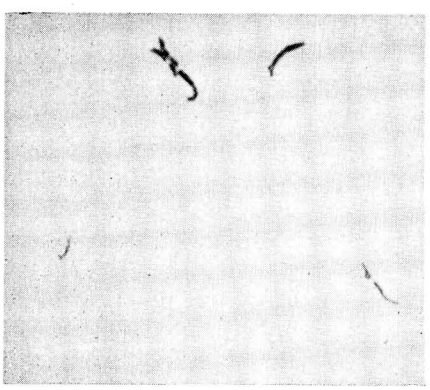
A brief summary of the characteristics of each of these five species of bacteria follows: RO-6TBR was a medium-size, Gram positive elongated anaerobic diplococcus that sometimes occurred in short chains. It was isolated from low dilutions of rumen contents from almost all cattle on all types of rations. This organism grew the most rapidly of any of the rumen organisms in the primary cultures and produced a heavy turbidity and a low final pH. Warburg studies showed that the organism was a homo-fermentative bacterium producing lactic acid from glucose. It produced acid in all of the carbohydrate media tested and grew in litmus milk, producing an acid reduced curd. It was not proteolytic. None of the B vitamins was used, but small amounts of biotin were synthesized.

TABLE 3

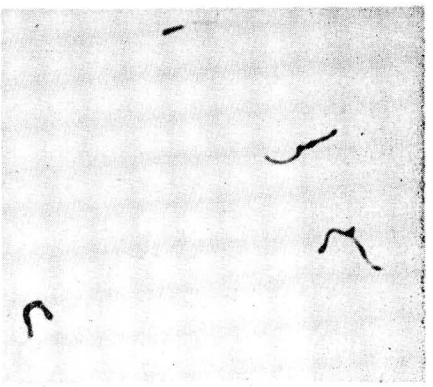
Data on number and distribution of isolations of five rumen organisms

Designation of culture	Range of dilutions	No. of times isolated and recognized	Age of animal and ration	Geographical locations
RO-6TBR	Usually 10^{-6} and 10^{-7} Seldom 10^{-8}	Over 3000	Almost all animals on all rations	New York, Tenn., and Ohio
RO-L_1	Usually 10^{-8} and 10^{-9}	Over 50	Calves on practical ration and mature sheep on purified rations	New York, Tenn., and Ohio
RO-L_2	Usually 10^{-7} and 10^{-8}	Over 50	" " "	New York and Ohio
RO-L_3	Usually 10^{-8} and 10^{-9}	Over 50	" " "	New York and Ohio
RO-T_1	Usually 10^{-8} and 10^{-9} or 10^{-10}	Over 50	Same as RO-L_1 plus immature ruminants on a deficient ration	New York, Tenn., and Ohio

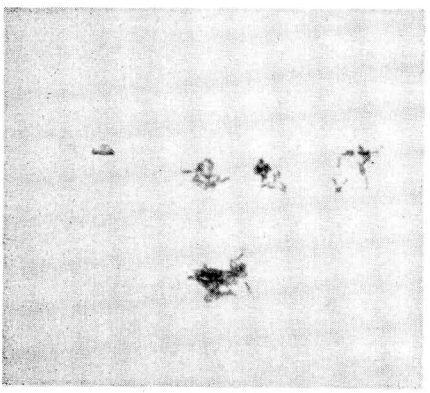
RO-T_1 was a long, thin, threadlike Gram negative rod with Gram positive granules. It was isolated from the higher dilutions of rumen contents of calves and from adult ruminants on purified rations, especially those in poor physical condition. It was a non-spore-forming anaerobe, which grew rapidly in rich organic broth, producing a slight turbidity and gas. The pH was lowered to about 4.5, as the result of lactic acid production. Warburg studies showed that the organism was heterofermentative. The gas produced from glucose fermentation was at least partly CO_2 and the acid was lactic. All of the carbohydrates tested were fermented, producing acid and gas. Growth in litmus milk produced acid reduced curd, and the organism was not proteolytic. RO-T_1 synthesized none of the B vitamins tested for, but required pantothenic acid, biotin and B_{12}.



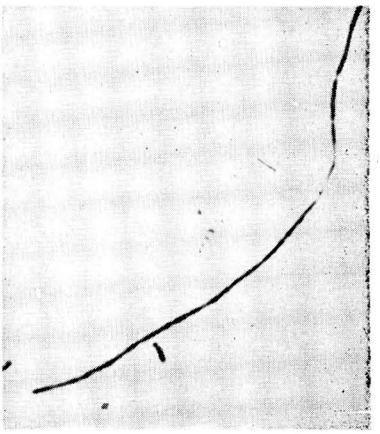
RO-L_1. Gram stain



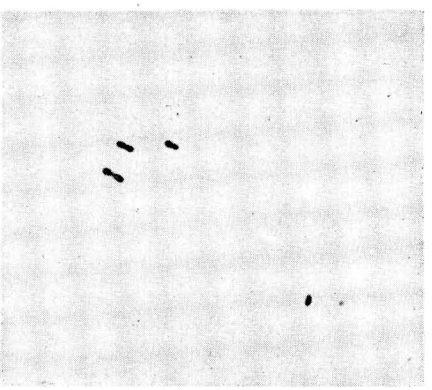
RO-L_2. Crystal violet stain



RO-L_3. Crystal violet stain



RO-T_1. Crystal violet stain



RO-6TBR. Crystal violet stain

FIG. 1. Photomicrographs of five cultures isolated from rumen contents. (×1920)

Although the three rods RO-L_1, L_2 and L_3 have a very similar physiology in many respects, they were distinguishable from each other morphologically. RO-L_1 was a Gram variable thick irregular staining pleomorphic rod, whereas RO-L_2 was a Gram variable poor staining pleomorphic rod characteristically showing branching and granules. RO-L_3 was a Gram positive short slender rod, which frequently occurred in packets which are called rafts. These organisms usually were isolated from calves on practical rations or adult ruminants on purified rations. None of the rods produced spores. All three rods were anaerobes, grew rapidly and gave a heavy turbidity and usually a sediment in broth. They all lowered the pH to about 4.4 to 4.7, and Warburg tests showed that they were homo-fermentative organisms producing lactic acid from glucose. All three organisms produced acid in most of the carbohydrate broths tested with the exception that RO-L_1 and RO-L_2 usually did not produce acid in the starch media. All three produced an acid reduced curd when grown in litmus milk and none of the three was proteolytic. The greatest differences between these three organisms lay in their B vitamin requirements. RO-L_1 synthesized folic acid, but used slight amounts of pantothenic acid. RO-L_2 synthesized none of the B vitamins tested for, but used thiamine, riboflavin, panthothenic acid and B_{12}. In contrast, RO-L_3 required none of the B vitamins, but synthesized pantothenic acid and some B_{12}.

The results of the cellulose digestion studies carried out with these organisms are still incomplete and will be reported later.

DISCUSSION

The rumen is subjected to almost continuous bacterial contamination from outside sources, whenever food, water or other foreign matter is swallowed. It has been demonstrated, for instance, that aerobic contamination of the rumen reaches a peak of about 1 to 10 million organisms per gram of rumen contents directly after eating, but since the rumen is an anaerobic organ, these aerobic bacteria fail to establish in the rumen and rapidly decrease in numbers for several hours after eating (7). Since only bacteria which can live and grow in the rumen could contribute much to digestion of food in the rumen, it seemed essential to make sure that an organism isolated from the rumen is a true rumen inhabitant and not just an incidental organism. It is difficult to do this because the incidental organisms are much easier to culture than the true rumen bacteria.

Since the rumen is an anaerobic organ, it was not surprising to find that at least 99 per cent of the bacteria isolated from the rumen contents above the level of contamination (the millionth dilution) are obligate anaerobes. Anaerobiosis is considered to be the very important first criterion for judging the bacterium to be a true rumen organism.

Direct slide counts of the bacteria showed that there are at least 50 to 100 billion bacteria per gram of fresh rumen contents. It seems clear that an organism outnumbered by at least 500,000 to 1 could not be considered to have established itself in the rumen as an important, predominating organism, so bacteria present in less than a million per gram are not studied. This has the

added advantage of being above the contamination level of about 1 million per gram, reached a few hours after eating. The second criterion is to consider only bacteria present in the millionth dilution or above as true rumen bacteria.

In the course of these rumen studies screen tests have been set up in order to be able to recognize similar organisms. It is gratifying to notice that about 20 types of rumen bacteria were isolated repeatedly, and that about 85 to 90 per cent of the organisms isolated fall into one of these 20 or so groups, as a recognizable type. It is to be expected as the studies continue and the technics improve that more type cultures will be established. Since studies made of the rumen organisms are of value only if the findings can be applied to more than one animal, it is extremely helpful that similar organisms can be isolated repeatedly from the rumens of animals fed similar rations. This may not be entirely due to chance, since there are indications that there is a bacterial flora peculiar to the rumen. From these findings it does not seem unreasonable to set 10 separate isolations of a similar bacterium from at least two animals, as the third criterion for judging an organism to be a true rumen organism.

Rumen bacteriological studies have been carried out on cattle and sheep in several different localities. Again, fortunately, the same types of organisms have been isolated from the ruminants in various regions when these animals are maintained on a similar ration. This lends support to the theory that there may be a characteristic rumen flora which is rather widespread in occurrence, whenever feeding conditions are similar. The fourth criterion for establishing an organism as a true rumen organism is its isolation in at least two localities so that the possibility of cross contamination between animals may be eliminated.

In the studies of rumen bacteria, the organisms have been allowed to act on some substrates that would be found in the rumen either as part of the original ration or as a breakdown product of the ration. The 20 type cultures characteristically produced end-products that are found in the rumen. This tends to confirm the idea that these organisms can function in the rumen, converting food into end-products that are known to be found in the rumen, and strengthens the probability that these are true rumen organisms. The production by the organism of end-products found in the rumen from substrates found in the rumen has been set up as the fifth criterion.

The five bacteria chosen to illustrate the type of experimental work carried out to set up these criteria were selected because these bacteria have a simple metabolism, quite similar to each other. Limitation of space made it seem unwise to try to discuss all 20 type cultures, although all types of cultures were used in establishing these criteria. The five bacteria described in this paper were all fast-growing carbohydrolytic bacteria that were lactic acid producers. RO-T_1 produced some gas in addition to lactic acid. These bacteria were found typically in the rumens of animals fed large amounts of available carbohydrate, and, therefore, should not be considered to be typical of rumen bacteria in general. For example RO-6TBR was found in almost all adult ruminants in the lower dilutions (10^{-6} and 10^{-7}) of rumen contents and increased directly in proportion to the amount of grain fed. In ruminants fed fattening rations, this

organism frequently was found in the billionth and sometimes the 10-billionth dilution of rumen contents. The RO-L types of rods and RO-T_1 type seldom have been isolated from the rumen of adult animals on practical rations, but occurred regularly in high dilutions in calves and in adults on experimental rations high in available carbohydrate. It is interesting to note that these RO-L types are similar in their physiology in many ways, but they differ in the synthesis or use of the B vitamins. This characteristic is, of course, of extreme importance to the host animal's welfare. The presence of a non-synthesizing, vitamin-requiring predominant bacteria in an animal's rumen may seriously interfere with the progress of the ruminant, unless the B vitamins are supplied in abundance by the ration or by other organisms.

SUMMARY

About 5,000 isolations of bacteria from the rumens of approximately 350 cattle and sheep from several herds in three states have been studied.

The data from these studies have been used as the basis for setting up these five criteria from judging true rumen bacteria: (a) Anaerobiosis; (b) presence in numbers of 1 million or more per gram of fresh rumen contents; (c) isolation of a similar type bacterium at least ten times from at least two animals; (d) isolation from animals in at least two geographical locations; and (e) production by the organism of end-products found in the rumen from substrates found in the rumen.

A description of some physiological characteristics of five rumen bacteria is included.

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ASSOCIATION ANNOUNCEMENT

Registration and housing headquarters will be in University of Tennessee Memorial Auditorium from 10:00 a. m., to midnight, June 5 and again June 6–8.

Housing facilities will be available in University of Tennessee dormitories, hotels and tourist courts in or near Knoxville.

The University Cafeteria will be open Tuesday evening, June 5, through noon, Friday, June 8. Other eating places in the vicinity of the University will be open as well as numerous places in the city, a mile away.

Knoxville is on main highways running east and west and north and south. It is served by the main routes of the L & N Railroad and the Southern Railway. There are excellent air transportation facilities served by American, Delta, and Capital airlines.

Announcements of the meeting will be sent to individual members of the Association, including a card for advanced registration and room reservations.

Projectors will be available in all lecture rooms for showing 3.25 x 4 in. and 2 x 2 in. slides.

Groups wishing rooms for sectional, committee or other special meetings should contact W. W. Overcast, McCord Hall, University of Tennessee, Knoxville, Tenn.

Provisions can be made for special breakfasts, luncheons or dinners by writing to T. W. Albrecht, McCord Hall, University of Tennessee, Knoxville, Tenn., by May 20.

Information on points of interest in Tennessee, scenic, historical and recreational, may be secured by writing the State Department of Conservation, Nashville, Tenn., and the Tourist Bureau, Knoxville, Tenn.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

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

BOOK REVIEWS

173. Official Methods of Analysis of the Association of Official Agricultural Chemists. 7th ed. Association of Official Agricultural Chemists, Washington 4, D. C. 910 pp. $10.00. 1950.

In addition to the latest procedures of this group for the analysis of dairy products, methods for analysis of a wide variety of materials of interest to people in dairying are presented. Those procedures which have been adopted since the issuance of the 6th edition in 1945 are included; a number of procedures also have been deleted.

The test for water-insoluble acids is included as official and the test for volatile acids (butyric acid test) as first action, both being for use on butter and cream. The test for fecal material and the lintine disc procedure for extraneous matter in butter are eliminated. The acidity test for milk, employing dilution with an equal quantity of water, was made official.

Attention has been given to reduction of the space required for each test by increased use of abbreviations and considerable brevity of presentation. This book should be in every control laboratory. F. E. Nelson

174. Breeding and Improvement of Farm Animals. V. A. RICE and F. N. ANDREWS. 4th ed. McGraw-Hill Book Co., Inc., New York, N. Y. 787 pp. $7.00. 1951.

This 4th revision of the book originally published by the senior author 25 yr. ago apparently is intended as a full year text to include all that which junior or senior students in agriculture need of embryology, physiology of reproduction, genetics and animal breeding. The organization of the 3rd ed. is retained but new material has been included, particularly in the section on Mechanisms of Reproduction and The Art of Breeding. A comprehensive chapter in the latter section, Selection in Meat Animals, by E. J. Warwick, presents an organized view of the results of recent research in this field. One little-needed chapter on statistical analysis in the 3rd ed. has been dropped, probably to the dismay of some breeders who will find statistical terminology more liberally sprinkled through the later chapters of this 4th ed. Some errors in the 3rd ed. are repeated in the 4th. For example, the method (p. 604) of evaluating the breeding value of a bull is incorrectly stated as being the average of what is expected of a bull's daughters. In addition, the assumption of complete heritability made by Wright in deriving the method in 1931 is not mentioned. However, almost all of the ample evidence accumulated in the last 15 yr. indicates that heritability of butterfat production, for example, is about 20%. The "cow family" philosophy continues to be overemphasized as regards dairy cattle breeding, although rational application of the genetic principles presented earlier in the book shows the underlying fallacy as applied to slowly reproducing species. The optimistic comments concerning the genetic impact of artificial breeding appear overdone, chiefly because this is a field where genetic emphasis and guidance are sorely needed. As far as concerns the dairy cattle of the future, the net result of using progeny-tested bulls in A. I. Centers may be little more than to prevent their use in the purebred herds which supply the next generation of bulls. The wisdom and economy of including about 50 of the some 70 animal pictures seems doubtful, although this seems to be the accepted style in popular books on animal husbandry subjects. L. N. Hazel

175. Amino acids and proteins. Theory, methods, application. D. M. GREENBERG, editor. Charles C. Thomas, Springfield, Ill. 950 pp. $15.00. 1951.

The chapters include Properties of amino acids, by E. E. Howe; Methods for the determination of amino acids, by Harold S. Olcott; The preparation of amino acids and polypeptides, by S. Archer; The synthesis of labeled alpha amino acids, by James C. Reid and Bert M. Tolbert; Isolation of amino acids, by Max S. Dunn and Louis B. Rockland; Classification, purification and isolation of proteins, by Harry L. Fevold; Determination of the molecular size of proteins, by Harold P. Lundgren and Wilfred H. Ward; Amphoteric properties of amino acids and proteins, by David M. Greenberg; Criteria of the purity of a protein, by Choh Hao Li; Chemical reactions of proteins, by Heinz Fraenkel-Conrat; Nutritional applications of the amino acids, by H. J. Almquist; The chemistry of antibodies, by Dan H. Campbell and Frank Lanni; Biochemical applications of proteins and peptides, by David M. Greenberg; The metabolism of amino acids and proteins, by Harold Tarver.

Detailed tables of contents at the beginning of each chapter, a good over-all index, many extensive tables, liberal use of formulae and extensive references to the original literature add much to the usefulness of the book. This is a valuable reference book for all who have an interest in these types of compounds. F. E. Nelson

176. Ice cream and other frozen desserts. J. H. FRANDSEN and D. H. NELSON J. H. FRANDSEN, Amherst, Mass. 318 pp. $5.25. 1950.

This is a concise up-to-date book on ice cream and related frozen dairy foods written in non-technical language. The book is designed to supply essential information needed by the plant manager, ice cream maker, retail store operator and ice cream salesman. Subdivision of subject matter into 25 individual chapters, each of which is described briefly in the contents, is of assistance to the reader in locating any specific information in which he may be particularly interested. The authors have drawn on their experiences accumulated over many years' association with students and with those engaged in industry to present in a concise manner subject matter relating to the production and sale of ice cream and related products and their value as a food. The contents include chapters relating to: The history and development of the industry, food value, classification, composition and properties, ingredients, stabilizers and emulsifiers, flavors and colors, calculations, making and processing of the mix, freezing, packaging, hardening and shipping, high fat and high serum solids ice cream, ices and sherbets, fancy ice creams and novelties, ice cream mix formulas, defects, scoring and grading, sanitization, refrigeration, laboratory tests, sales outlets, formulas for soda fountain use, and reference material. There are 48 illustrations and 42 tables. Simple methods which are fully explained and illustrated are presented for the calculation of: ice cream mixes, the restandardization of ice cream mixes, and overrun and weight of ice cream. The retail store operator and ice cream salesman will find much helpful information in the chapters dealing with retail outlets, formulas for soda fountain use, special ice creams and novelties and in the reference material. The text is well written, indexed and illustrated. It should meet the needs of the practical man who is interested in learning how to make frozen dairy foods, how to sell them and their value as a food. W. J. Caulfield

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

177. The control of Streptococcus agalactiae infection in herds by means of therapeutic treatment. S. J. EDWARDS and J. I. TAYLOR. Vet. Record, **61,** 47: 780–783. 1949.

One herd of 70 Ayrshire cows that had been tested regularly for *Str. agalactiae* infection for several years and was machine-milked and stripped, with precautions taken to prevent the spread of mastitis, was studied first. Just prior to treatment, 54 cows were shown to be infected, 22 showing clinical signs of mastitis. Infected cows were treated for 4 d. with 50 ml. of a 30% sulfanilamide emulsion in an oil-water base per quarter, and 61% of the infected cows responded to this treatment. Another treatment was given 2 wk. later using up to 8 daily injections if daily samples indicated infection was still present. Sulfanilamide was finally effective in 85% of infected cows treated. Remaining infected cows plus newly infected cows were treated with penicillin in water, using 4 daily injections of 100,000 units each per quarter. This treatment was successful and, with the exception of 3 new cases, the herd remained free of *Str. agalactiae* infection for over 2 yr.

Five more herds infected with *Str. agalactiae* were included, in which the incidence of infection was 21%. Treatment consisted of 4 daily injections of 40,000 units of penicillin in a 4.5% beeswax-oil base. In the first course of treatment, 31 of the 55 infected cows were treated and 24 served as controls. All but 2 of the treated cows responded, and in subsequent treatment of the 24 controls in which infection remained, all but one responded. Herds remained in the experiment for 20 mo. and in 2 herds the infection was eradicated, while in a 3rd it was reduced to a single cow that did not respond to successive treatments. In the 2 remaining herds the incidence was lowered for a short period, but rose repeatedly. Teat lesions and no precautions to prevent the spread of infection were believed to account for this.

The authors concluded that some cows infected with *Str. agalactiae* resist treatment from both sulfanilamide and penicillin, with penicillin being most effective. R. P. Niedermeier

178. A comparison of ante-mortem and post-mortem findings in bovine mastitis. D. MCFARLANE and P. S. BLACKBURN. Vet. Record, **61,** 49: 807–810. 1949.

This experiment was designed to obtain a comparison between the diagnostic value of cell count and culture tests and to determine if typical mastitis pathogens are present in quarters producing milk of high cell content but free of pathogens. Fifty-four quarters with a record of high cell counts were examined and ante- and post-mortem data are presented. Cell counts of an animal were considered positive if the average count of the mid-lactation samples was over 100,000/ml. Post-mortem histological results were considered positive if there was evidence of what the authors term progressive or dormant mastitis. Pathological findings were present in 92% of the quarters with high cell counts, indicating that cell counts are a reliable method of diagnosis for mastitis.

In 39 quarters, a comparison was made between ante-mortem milk cultures and post-mortem udder tissue cultures, and 80% agreement was obtained. Further comparisons resulted in 56% agreement between ante-mortem culture tests and post-mortem histological examination, and 62% agreement between post-

mortem tissue culture tests and histological examination. In 39% of the histologically positive quarters no typical mastitis organisms were found in milk ante-mortem or in the tissues post-mortem, indicating a mastitic condition can exist even when culture tests are negative.

The authors conclude that culture tests of the milk are not as reliable for diagnostic work as cell count tests, and a non-specific form of bovine mastitis often may be present, although mastitis organisms or other pathogens are not present in the milk or udder tissues. R. P. Niedermeier

179. The ring test for bangs. Anonymous. Butter, Cheese & Milk Prod. J., **41,** 11: 32–33, 38–39. Nov., 1950.

The ring test was developed in Germany in 1937. When used in conjunction with the standard blood or agglutination test, it is a big help in locating brucellosis. The principle of the ring test is that a colored antigen is added to sample of milk being tested; in milk from infected cows the colored antigen collects in the fat droplets and rises to top forming a blue ring. The skim portion remains white. In negative milk, the colored antigen remains in the skimmilk portion. The test works most accurately on mixed milk of 5–10 cows. Thus, it lends itself for use at receiving stations. Samples should be taken from milk cans rather than from mixed milk in weigh can. Positive ring tests must be followed by confirming blood tests on suspected herds. False-positive ring tests may result from testing milk of cows early or late in lactation, milk from vaccinated animals and milk from self-cleansed or ceased reactors. Comparison of the milk and cream ring test with blood tests on over 6,000 herds indicated the ring test to be 75% efficient in locating infected herds. H. E. Calbert

180. The occurrence of Vibrio foetus in aborted material derived from cows inoculated with S. 19 Br. abortus vaccine. W. R. Wilson, Reading Cattle Breeding Centre, and A. McDiarmid, Agr. Research Council, Field Sta., Compton, Berkshire. Vet. Record, **62,** 40: 589–591. 1950.

This report concerns a study of causes of abortion in 20 herds inoculated against *Br. abortus.* The 32 abortions studied included bacteriological and microscopic smear examinations of foetal stomach contents, cotyledons, colostrum and blood. *Br. abortus* was found in 3 cases in 3 different herds, all being other than st. 19 infections. The only other identified organism of significance was *V. foetus,* which was found in 7 animals representing 3 different herds. All of *V. foetus* abortions occurred between 5th and 8th mo. of pregnancy. R. P. Niedermeier

BUTTER

O. F. HUNZIKER, SECTION EDITOR

181. Sanitation and its relation to "cheesy" flavor in sweet cream butter. B. M. Zakariasen. Butter, Cheese, & Milk Prod. J., **41,** 11: 24–25, 47. Nov., 1950.

An off-flavor known as "surface taint" or "cheesy flavor" often results from poor keeping quality in a sweet cream butter. This defect was noted in butter stored for 2 wk. or more at 45–50° F. Records indicated that this butter was made from a high quality cream and was produced under excellent sanitary conditions. Treatment of the wash water with 15 ppm. of chlorine in form of sodium hypochlorite eliminated this defect. To guard against surface taint in sweet cream butter it must be produced from high quality cream, under good sanitary conditions and washed with "safe" water.

H. E. Calbert

182. The WIA test for butter. T. K. Forster. Butter, Cheese & Milk Prod. J., **41,** 10: 36, 69–70, 72. Oct., 1950.

The water-insoluble acids (WIA) test was developed by Hillig of the Federal Food and Drug Administration, for use in determining in a sample of butter the quality of cream from which it was made. Butter made from fresh sweet cream or from clean sour cream usually will contain less than 200 mg. of WIA/100 g. of fat. Butter made from high-acid cream or cream in advanced stage of decomposition will have a WIA content of 500 mg. or more /100g. of fat. The WIA content of butter closely parallels that of cream from which it is made. The presence of a high WIA content in a cream is caused by a chemical reaction promoted by enzymes known as lipases. These lipases originate as a result of growth of bacteria, yeasts and molds. Washing butter granules, vacuum pasteurization and neutralization will not alter the WIA appreciably. There is no significant increase in WIA in butter stored for 5 mo. at 0° F.: 5-mo. storage at 40° F. caused an increase in WIA only in butter samples in which mold developed. H. E. Calbert

183. Etudes rhéologiques sur le beurre (Rheological studies of butter). M. Beau. Lait, **30,** 299–300: 593–608. Nov.–Dec., 1950.

A review of the work of Mohr (Die Milchwissenschaft 1948, 1949) on certain physical properties of butter is presented. Such factors as tensile strength, resistance to cutting, penetrability, viscosity, appearance on melting, etc. were studied. The investigation considered butter prepared by the Fritz and Alfa processes, as well as conventionally churned samples. A number of original devices for measuring the physical properties of butter are illustrated and described. In general, Alfa process butter was considered to have superior properties. Presumably, this results from the absence of any large air cells or buttermilk droplets in such butter. Consequently, it has a relatively high resistance to deformation, disintegration, cutting and oiling-off when compared with Fritz process or conventionally churned butter. The physical properties of Alfa process butter appear particularly desirable from the viewpoints of butter manufacture, distribution and consumption. S. Patton

Also see abs. No. 194.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

184. Cheddar cheese defects. J. A. NELSON. Butter, Cheese & Milk Prod. J., **42,** 1: 28–29. Jan., 1951.

Three essentials in the manufacture of high quality cheddar cheese are high quality milk, a good active culture and good workmanship. Knowledge of manufacturing technics is helpful in understanding some of the cheddar cheese defects. The "time schedule" or "clock method" is the most satisfactory way of making cheddar cheese. The most prevalent flavor defects are unclean, bitter and acidy. Open texture is the most common body and texture criticism. Acid cut color accounts for most color defects in cheddar.

Unclean flavor is caused by poor quality milk, poor starter culture and improper amount of "wet" acid development during manufacturing. Abnormal protein breakdown is responsible for bitter flavor in the cheese. Acidy flavors can be eliminated by proper acid development in the manufacturing process. Careless handling of finished cheese may cause scaly paraffin, mold and soiled surfaces, cracked rind, rind rot and misshapen cheese. H. E. Calbert

185. Apparatus for manufacturing cheese and the like. B. S. HARRINGTON (assignor to Armour and Co.). U. S. Patent 2,536,054. 7 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 82. 1951.

This consists of a perforated horizontal cylinder suspended on 2 endless chain belts from a rotating shaft. Milk, suitably coagulated, is introduced into the cylinder, which is caused to rotate. The whey drains through the perforations and is collected in a trough. The curd particles are kept in motion by rolling within the cylinder and by agitating action of breaker arms which are turned within the cylinder. R. Whitaker

186. Rectangular cheese hoop. N. J. PETERS (assignor to Damrow Bros. Co.). U. S. Patent 2,538,379. 1 claim. Jan. 16, 1951. Official Gaz. U. S. Pat. Office, **642,** 3: 893. 1951.

Structural features are given for an improved cheese hoop of rectangular shape.

R. Whitaker

187. Cheese-cutting device. H. A. OLANDER. U. S. Patent 2,538,426. 19 claims. Jan. 16, 1951. Official Gaz. U. S. Pat. Office, **642,** 3: 905. 1951.

A small cheese cutter or slicer of the taut wire type is described. R. Whitaker

188. Art of packaging cheese. E. E. ELDREDGE (assignor to the Borden Co.). U. S. Patent 2,540,815. 2 claims. Feb. 6, 1951. Official Gaz. U. S. Pat. Office, **643,** 1: 254. 1951.

Green cheese is wrapped in 2 layers of a dry sheet wrapping material which is impervious to moisture and gases and which shrinks on heating.

R. Whitaker

189. Mechanism for packaging cheese and other viscous material. H. A. STINE (assignor to Kraft Foods Co.). U. S. Patent 2,540,557. 13 claims. Feb. 6, 1951. Official Gaz. U. S. Pat. Office, **643,** 1: 186. 1951.

Structural details are given for a jar filler for viscous materials like process cheese, in which the filling spout moves into the empty jar and moves out as it is filled with the product.

R. Whitaker

Also see abs. No. 219.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

190. Reconstituted milk from low heat non-fat dry milk solids and dry milk fat. A. O. DAHLBERG. Butter. Cheese & Milk Prod. J., **41,** 10: 26–27, 58, 60. Oct., 1950.

A reconstituted milk suitable for beverage purposes can be made from low heat NFDMS and anhydrous or dry milk fat (DMF). DMF is made by (a) reseparation of pasteurized 40% cream to heavy 80% cream, (b) breaking emulsion of heavy cream, (c) separation of fat and milk serum by gravity, (d) removing curd and moisture by centrifuging and/or vacuumizing, (e) quick cooling of milk fat and (f) packing in sealed tins.

The reconstituted milk is made by dissolving NFDMS in pure clean water. Melted DMF is added to the above mixture when temperature is above the melting point of fat. The reconstituted milk is pasteurized, homogenized and cooled with a surface or plate cooler. The finished product has a flavor similar to that of homogenized market milk of the same butterfat content. It is free of speckiness or chalkiness.

H. E. Calbert

191. Method of preserving spray-dried food products. P. F. SHARP and A. J. WASSON (assignors to Golden State Co.). U. S. Patent 2,541,441. 6 claims. Feb. 13, 1951. Official Gaz. U. S. Pat. Office, **643,** 2: 519. 1951.

To reduce the oxygen content, spray-dried milk powder is placed in bulk in a tank. The air is evacuated and the vacuum released with an inert gas. The oxygen diffuses from the interior of the powdered particles and is replaced by the inert gas which surrounds the particles. The inert gas containing the oxygen in the tank is removed from time to time by vacuum and replenished with a fresh supply of inert gas, until the oxygen content of the discharged gas is reduced to 1–3% when discharged at the rate of 0.01–0.05 ft.^{3}/hr./100 lb. powder The deoxygenated powder then is packaged in sealed containers under non-oxidizing conditions.

R. Whitaker

192. Pyrogene stoffen in melk en melk poeder (Pyrogenic substances in milk and milk powder). H. VAN GENDEREN, Ryksinstituut voor de Volksgezondheid, Utrecht, Holland. Neth. Milk and Dairy J., **4,** 4: 299–308. 1950.

The first part is a review on pyrogenic substances in general. These substances, produced by bacteria, cause fever when introduced into the blood. Several samples of dried milk were tried on rabbits. Fever was obtained with 0.02–0.1 ml. reconstituted milk. Differences were relatively small, within a factor of 10. No correlation was found with bacteriological quality. Some of the samples that might have caused food poisoning did not show particular activity. However, no conclusion could be drawn about this, as the toxicity of the samples was not checked and the number of samples was too small. Fresh milk was pyrogenic to about 0.5 ml. Heat action decreases pyrogenic substances.
A. F. Tamsma

193. Noncurdling high calcium milk product and method of producing same. L. S. GAUR and M. P. GERBER (assignors to M and R Dietetic Labs., Inc.). U. S. Patent 2,541,568. 18 claims. Feb. 13, 1951. Official Gaz. U. S. Pat. Office, **643,** 2: 552. 1951.

The Ca/P ratio of milk is increased from the usual 1.3: 1 to a 2.43: 1 ratio by the addition of an edible Ca ion-containing compound. Then a non-toxic compound containing citrate ions is added in an amount to completely destroy the curd-forming properties of the milk. Following the further addition of vegetable and animal fats and lactose, the product is condensed and dried to a powder. R. Whitaker

194. Process of treating milk oil. C. E. NORTH. U. S. Patent 2,540,830. 7 claims. Feb. 6, 1951. Official Gaz. U. S. Pat. Office, **643,** 1: 259. 1951.

Milk fat, at a temp. between its melting point and 212° F. is expelled through perforations into acidified water and allowed to rise in the form of droplets to collect on top as an oil layer. Following this washing treatment the milk fat is dewatered in a centrifuge. R. Whitaker

195. Process for canning and sterilizing food products. H. L. SMITH, JR. and C. O. BALL (assignors to Food Processes, Inc.). U. S. Patent 2,541,113. 19 claims. Feb. 13, 1951. Official Gaz. U. S. Pat. Office, **643,** 2: 432. 1951.

Foods are heated rapidly in bulk for a time and temp. equivalent to 50–97% complete sterilization and then cooled to a point above the boiling point where it is filled into containers at super-atmospheric pressure. The sealed containers then are maintained at the filling temp. and under pressure for a sufficient period of time to complete sterilization. R. Whitaker

196. Concentrating whey for feed. A. H. STEVENS. Butter, Cheese & Milk Prod. J., **42,** 1: 32–34, 36–38. Jan., 1951.

Two pilot-plant types of equipment were devised for concentrating whey in small cheese factories. The first was a submerged combustion unit utilizing a mixture of air and natural gas as the heating medium. With this type of unit, 0.60 lb. of water could be evaporated/ft.^{3} of methane gas. The whey condensed by this method was suitable only for animal feed. Spraying whey into heated air in a cone similar to a cyclone spray drier was used in concentrating the whey by the second method. A continuous operation was utilized, resulting in a product containing 33% solids. The quality of the product produced by the latter method compared favorably with that condensed under vacuum. H. E. Calbert

197. Method of treating casein threads, fibers and the like. L. MAASKANT (assignor to American Enka Corp.). U. S. Patent 2,539,958. 7 claims. Jan. 30, 1951. Official Gaz. U. S. Pat. Office, **642,** 5: 1447. 1951.

Resistance to hot acid dyes is imparted to casein fibers by treatment with 6 parts of formaldehyde to 1 part of resorcinol, chloro-, lower alkoxy- and carboxy-substituted resorcinol.
R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

198. Bacteriophage — the invisible enemy of starters. P. R. ELLIKER. Butter, Cheese & Milk Prod. J., **41,** 12: 28–30, 46. Dec., 1950.

Bacteriophage is a virus that attacks and destroys bacterial cells. Its action on lactic acid bacteria results in partial or complete stoppage of acid production by the starter culture as well as frequent damage to dairy products. The most frequent outbreak of "phage" has been in cheddar cheese plants. However, it has been encountered in plants manufacturing other types of cultured dairy products. The most pronounced starter failures due to bacteriophage usually occur with single strain rather than mixed strain starters. A discussion of various properties of phage as to size, shape, optimum temperature of growth, rate of reproduction and resistance to heat is given. Two methods of combating an attack of phage are: (a) changing to another strain or strains of starter culture, (b) a thorough plant clean up followed by sanitizing all equipment with solution containing 500 ppm. hypochlorite. H. E. Calbert

199. Zuurvorming en aromavoring in yoghurt (Acid production and aromaformation in yoghurt). J. W. PETTE and H. LOLKEMA, Ned. Inst. voor Zuivelonderzoek, Hoorn, Holland. Neth. Milk and Dairy J., **4,** 4: 261–273. 1950.

Yoghurt is considered as a mixed culture of *S. thermophilus* and *L. bulgaricus,* with symbiotic and antibiotic action between these two kinds of bacteria. The acid production was followed together with the numbers of different bacteria. In the first phase there is a high rate of development and acid production of *S. thermophilus; L. bulgaricus* develops later on.

The flavor consists of 2 main components, the lactic acid and the aroma-substance(s). As far as the acidity is concerned, at the time of consumption 85–90° D. (or about 95–100 ml. 0.1 *N* NaOH/100g.) was found to be most favorable for flavor. The typical yoghurt aroma-substances are produced by *L. bulgaricus* only at higher acidities. These substances could be isolated via

2,4-dinitrophenylhydrazone. However, when tried, acetaldehyde did not show yoghurt aroma. It is suggested that acetaldehyde comes into existence simultaneously with true yoghurt aroma.

Flavor defects are "too acid" or "flat" when the acidity is too low. "Unclean" and "bitter" can be found at various acidities and are caused by use of milk of bad quality or by use of a yoghurt culture containing strains causing bitterness.
A. F. Tamsma

200. A laboratory program for small dairies. H. WATROUS, JR., Penn. State College. Am. Milk Rev., **12,** 3: 18–20. Mar., 1950.

The program of the laboratory technician should include tests of the raw milk supplies, namely periodic examination of all incoming raw milk, low thermoduric bacterial count as measured by the laboratory pasteurization test, sediment test, fat test and lactometer reading, checking flavor and smell, and checking for conformity to temperature requirements. Laboratory tests relative to plant control should include daily checks on all processed products. Standard plate count and coliform determinations should be made on milk, cream and chocolate milk, and in some areas microscopic examinations are used for pasteurized milk and cream. A bacteriological line test at regular intervals will aid in detecting possible points of contamination, as will sterility tests for bottles, caps and cans. Fat tests and the phosphatase test should be run daily on all pasteurized products. Other inspections of processed products should include sediment determinations of milk and cream, cream volume in pasteurized milk, homogenization efficiency of homogenized milk, viscosity and whipping ability of cream, acidity, viscosity and absence of whey-ing off in buttermilk, and organoleptic examination of all products.

The technician also should propagate cultures for cultured products, select proper cleaners and check frequently to insure maintenance of proper temperatures in bottle and can washers, supervise cleaning and sterilizing of plant equipment and inspect the physical premises and all equipment after the daily clean up. D. J. Hankinson

201. Virulence du mycobacterium tuberculosis dans le fromage blanc (Virulence of M. tuberculosis in cottage cheese). LONCIN and J. GEAIRAIN. Lait, **30,** 299–300: 608–612. Nov.–Dec., 1950.

Much of the cottage cheese of France is prepared on the farm from raw milk. Considering the comparatively high incidence of bovine tuberculosis, such cheese represents a very definite public health hazard. To demonstrate the point, the author prepared cottage cheese from milk contaminated with milk from a tubercular cow. Guinea pigs, inoculated at periodic intervals with cell concentrates from the cheese, were all positive up to 14 d. of age of the cheese. The presence of *M. tuberculosis* in these animals was confirmed after death or sacrifice. After 2 wk., the cheese commenced to undergo alkaline putrefaction and 4 guinea pigs inoculated with material from the cheese at 17 d. of age gave negative or doubtful results. It is suggested that putrefactive organisms produce materials with antibiotic activity toward *M. tuberculosis.* S. Patton

202. Medium for assay of vitamins with lactic acid bacteria. L. M. FLYNN, V. B. WILLIAMS, B. L. O'DELL and A. G. HOGAN, Univ. of Missouri, Columbia. Anal. Chem., **23:** 180–185. 1951.

An easily prepared and flexible medium was devised for use in microbiological assays of folic acid, riboflavin, nicotinic acid and B_{12} activity. The medium was easily assembled from commercially available constituents, with only one adsorption to purify the casein hydrolyzate being required. The medium supported growth for *Lactobacillus casei, Streptococcus faecalis, L. arabinosus* and *L. leichmannii,* all of which are used for assays. Growth was measured either turbidimetrically or acidimetrically. Curves for standard cultures and for unknowns are parallel straight lines when responses are plotted against doses on a log-log basis. B. H. Webb

Also see abs. no. 181.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

203. Determining the butterfat content of milk. P. SCHAIN. Halloran Vet. Admin. Hosp., Staten Island, N. Y. Milk Dealer, **40,** 3: 49–50, 54–58. Dec., 1950.

A detailed description of a single solution detergent method for determining the butterfat content of milk is given. The advantages of the method are: (a) It is far simpler than any of the existing methods and is more accurate than either the Babcock or the Gerber tests. (b) Neither a centrifuge nor any other expensive laboratory equipment is used. The required apparatus consists of a graduate, a pipette, a Babcock bottle and a pot to boil water in. (c) No sulphuric acid or other caustic material is used in the test. The single reagent, a "soapless soap", is harmless to skin and clothing. (d) The procedure has none of the technical difficulties embodied in the tests now in use. It may be performed easily and accurately by the average untrained individual who can follow simple instructions. C. J. Babcock

204. Babcock test mixer. G. F. MASSEY. U. S. Patent 2,539,851. 3 claims. Jan. 30, 1951. Official Gaz. U. S. Pat. Office, **642,** 5: 1420. 1951.

A motor-driven rack holding a number of Babcock test bottles is given a rotary motion which rapidly mixes the milk and acid. R. Whitaker

205. Determination des teneurs en vitamins C, A, B_1, B_2 de differents laits de femme (Quantitative determinations of vitamins C, A, B_1 and B_2 in samples of mother's milk). L RANDOIN and A. PERROTEAU. Lait, **30,** 299–300: 622–629. Nov.–Dec., 1950.

Ten samples of mother's milk were assayed, by conventional chemical methods, for vitamins C, A, B_1 and B_2 after collection, pasteurization

and 1 wk. and 4 wk. of cold storage. Wide variations between samples in the initial values of all vitamins were observed. Rather drastic losses (up to 100%) in vitamin C were experienced as a result of pasteurizing and storage of the milk. Appreciable losses of B_1 (average of 41%) were noted; the bulk of this loss seemed to have resulted from pasteurization. Vitamins A and B_2 demonstrated good stability, there being a loss of 8–10% of these during the 4-wk. period.

S. Patton

206. Buffered filter paper chromatography of the amino acids. E. F. McFarren, Natl. Dairy Research Labs., Inc., Oakdale, L. I., N. Y. Anal. Chem., **23:** 168–174. 1951.

Each of 20 amino acids were separated from all others in a mixture by 1-dimensional chromatography using several solvents buffered at a selected pH. Improved separation may be obtained with 2-dimensional chromatography, but it produces poorly defined spots and irreproducible *Rf* values. Each amino acid in an unknown mixture may be separated into well defined spots and identified by the 1-dimensional method without resorting to special reagents to confirm the identity of 1 or more of the acids. The chromatograms were buffered by dipping the paper into buffer of the desired pH and molarity, then air-drying. The solvent then was equilibrated with the same buffer, rather than with water. Extensive data are given for variations in *Rf* values of amino acids with the pH of the buffers. *Rf* values of amino acids as a function of molarity of buffer, temperature and vapor content of the chamber are presented. All solutions should be adjusted to a pH between 5.5 and 7.5 before spotting and the room temperature should be controlled to + 1° C. B. H. Webb

207. Paper chromatography of amino acids. Effect of pH of sample. A. J. Landua, R. Fuerst and J. Awapara, M. D. Anderson Hospital for Cancer Research, Univ. of Texas, Houston. Anal. Chem., **23:** 162–168. 1951.

Many amino acids made good chromatograms when the pH of the solutions was adjusted before placing on paper. Included were the amino acids in acid protein hydrolyzates from which most of the acid had been evaporated. The spread of the spot and its position on the chromatogram were affected by sample pH. Individual amino acids and mixtures gave results of a similar nature. Many charts in which were plotted *Rf* vs. pH values of amino acids in solvents, phenol, 2,4-lutidine and 1-butanol were presented. B. H. Webb

208. Quick test determines dissolved solids. R S. Robertson and M. F. Nielsen, Nat Aluminate Corp., Chicago, Ill. Power, **95,** 2: 87. Feb. 1951.

A titration test and test procedure for the determination of dissolved solids is presented. Results compare favorably with gravimetric methods for solids in boiler water.

The water sample is treated with a portion on analytical grade Nacite HCR which converts dissolved water salt to corresponding acids which can be titrated with standard Na_2CO_3 solution. The amount of Na_2CO_3 solution used multiplied by a factor plus the total alkalinity (determined by usual means) equals the dissolved solids in grains per gal. H. L. Mitten, Jr.

Also see abs. no. 173, 175, 182, 193.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

209. Ice cream freezer. A. H. Wakeman (assignor to The Creamery Package Mfg. Co.). U. S. Patent 2,538,716. 1 claim. Jan. 16, 1951. Official Gaz. U. S. Pat. Office, **642,** 3: 980. 1951.

A horizontal continuous ice cream freezer having an angular-shaped dasher is described.

R. Whitaker

210. Methods of making piping flexibility analysis. T. E. Bridge, Tube Turns Inc., Louisville, Ky. Heating, Piping & Air Cond., **23,** 1: 136–139. Jan., 1951.

A step-by-step method for calculating flexibility of piping systems is presented along with equations, symbols and example problems.

H. L. Mitten, Jr.

211. Pumping milk and cream. E. L. Fouts, Univ. of Florida. Am. Milk Rev., **12,** 3: 24, 26, 28. Mar., 1950.

Pumps used for milk and other fluid dairy products may be divided into 2 general classes: (a) the positive displacement type, such as a piston or rotary and (b) the non-positive type, such as the centrifugal pump. The latter is used most commonly in milk plants.

The most important feature to be considered in the selection and use of a pump is its capacity. A pump that is too large will, because of excessive churning, shorten the cream line of the milk and, after some time in storage, a cream plug will result. Regulating valves may be placed in the line to control the milk flow but this often affects the body and texture of both milk and cream. Such a valve must be on the discharge side to minimize agitation of the product.

Temperature of the product being pumped is very important. The fat will churn out much less readily in a liquid state than in a solid or semi-solid state. Low temperatures also decrease the tendency for milk or cream to churn. Therefore, the milk or cream to be pumped must be at a temperature of either 38–45° F. or above 125° F.

Because of the tendency of milk to develop oxidized or metallic flavor upon exposure to certain metals, especially Cu and Fe, a stainless steel pump is best.

Where several pumps are used, it is best to standardize on the size and make of the pumps to facilitate keeping repair parts and replacements on hand. Vapor-proof and water-proof motors are now available in small sizes and are recommended for dairy plant use.

D. J. Hankinson

212. Pumping milk and cream. E. L. FOUTS, Univ. of Florida. Am. Milk Rev., **12,** 4: 24, 26, 74–75. Apr., 1950.

An automatic electric milk level control may be used in vats, tanks, cooler troughs, etc. to stop or start the pump to maintain the milk at the desired level. Such a control is not available on any milk bottling equipment but it may be installed at a very small expense, and will soon prove to be a great economy to minimize the loss of product.

Milk may be pumped several times without affecting its quality, providing great care is taken to have the milk at proper temperature. Pumping from surface cooler to bottler can be done without damage to the product, providing it is done within a few minutes after cooling and before the cream has had a chance to rise. Successful pumping of milk to the cooler and again from the cooler trough must be a continuous process, rather than an intermittent one.

Any pump must be completely dissembled after each day's run and thoroughly cleaned. A centrifugal pump may be left apart or reassembled for the next day. A positive pump must be handled with great care and must be reassembled immediately after cleaning to avoid physical damage.

Cold ice cream mix may be pumped into the freezer without damage to the mix, providing the pump is stopped and started as the mix is needed. D. J. Hankinson

213. Het beproeven van moderne pasteurizatietoestellen (The testing of modern pasteurization equipment). H. C. MULLER, N. V. Melkcentrale "Noord", Amsterdam, Holland. Neth. Milk and Dairy J., **4,** 4: 308–327. 1950.

Requirements of hygienists, food physiologists and dairymen for modern pasteurization equipment are listed, including the general setup, how it works, the way the temperature changes through the apparatus, materials and heating media employed.

In the testing procedure the minimum temperature-time combination required for safe pasteurization is important. This can be checked by number of microorganisms, presence or absence of *coli* and pathogenic organisms and peroxidase and phosphatase tests. The equality of the heating procedure for all milk particles is important too. Particles staying longer than the required minimum in the apparatus, may cause cooked flavor, decrease in vitamin content and decrease in heat transmission when precipitated on the walls. This can be checked by making a flow diagram. This should be done by alternating water and milk under pasteurization conditions. From fat determinations in sample series, the characteristics of the mixing zones can be found and the time differences for particles in the apparatus calculated. Figures for a modern apparatus are in the range 200 ± 5 sec.; for the part heater and holder, 40 ± 1 sec. In testing equipment, it is advisable to control the pasteurization at a series of different temperatures. In experiments of this kind with modern pasteurization equipment, the best results were obtained at low temperatures (around 70° C.). This was based on peroxide and phosphatase tests, number of microorganisms, *coli* test, methylene blue test, stability at 30 and 15° C., cream line formation, rennet coagulation and albumin denaturation.

A. F. Tamsma

214. Method of pasteurizing, sterilizing and steam distilling potable liquids. H. L. MURRAY (assignor to Murray Deodorizers, Ltd.). U. S. Patent 2,539,264. 5 claims. Jan. 23, 1951. Official Gaz. U. S. Pat. Office, **642,** 4: 1193. 1951.

Dairy and other fluid food products are sterilized and steam-distilled in this equipment, which consists of 2 chambers or vessels. In the first, the product is intimately mixed with steam under pressure and with sufficient water to reduce the steam temperature to that corresponding to the selected temperature. From the first vessel the heated product passes to the second where it boils under reduced pressure, the released vapors removing objectionable flavors from the product.

R. Whitaker

215. Method and installation for homogenization of liquids. O. E. FRÖDING (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,539,125. 12 claims. Jan. 23, 1951. Official Gaz. U. S. Pat. Office, **642,** 4: 1157. 1951.

Homogenized milk, having the fat in a very finely emulsified form, is produced by passing the homogenized milk through a centrifugal separator which separates it into 2 components, one containing very small fat particles and the other containing relatively large globules. The latter component is fed back into the suction line of the homogenizer for rehomogenization along with the incoming unhomogenized milk.

R. Whitaker

216. Why not hydraulics? H. P. FAUST, R. G. Wright Co. Am. Milk Rev., **12:** 5, 50, 51. May, 1950.

Because of the ever-present high moisture conditions in a dairy plant and the detriment such conditions cause to the machinery, a closed hydraulic system is much more efficient than any other power unit. Hydraulic applications already are on the market and offer many advantages. The dairy industry should make greater use of hydraulic systems.

D. J. Hankinson

217. Clean that crankcase—but. . . . W. N. DAVIS, Natl. Safety Council. Operating Eng., **4,** 1: 36–37. Jan., 1951.

The use of gasoline for washing and flushing refrigerating compressor crankcases is dangerous because (a) vapors are explosive, (b) vapors have a toxic effect on the respiratory system and (c) they burn hands and skin. Vapors flow rapidly along the floor and settle in low spots where they are extremely dangerous as an explosion hazard. Kerosene doesn't have the explosive potential of gasoline if kept below 100° F.

Carbon tetrachloride vapors are toxic and are very dangerous when used in a poorly ventilated area, so a forced-circulation blower should be used. Most petroleum solvents are flammable; however, there are many having a high flash point which can be used safely to flush crankcases. One of these consists of 25% methylene chloride. 70% Stoddard solvent and 5% perchloroethylene. Alkaline chemicals dissolved in water may be used if they are followed with a thorough warm water rinse. Time must be allowed for complete drying after the water rinse. H. L. Mitten, Jr.

218. Chemical and engineering unit operations review. Ind. Eng. Chem., **43:** 37–116. 1951.

This 6th annual review contains, among others, the following sections which may be of interest to dairy technologists: Centrifugation, J. O. Maloney; Crystallization, C. S. Grove, Jr. and J. B. Gray; High Vacuum Distillation, K. C. D. Hickman; Drying, S. J. Friedman; Evaporation, W. L. Badger and R. A. Lindsay; Ion Exchange, R. Kunin. B. H. Webb

Also see abs. no. 185, 220, 233, 242.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

219. Cheese factory operating costs. L. C. THOMSEN. Butter, Cheese & Milk Prod. J., **41,** 9: 32–35, 56–58, 60–62. Sept., 1950.

This is a review of published data available for determining cheese factory operating costs. It is not readily possible to convert this data for present day usage due to lack of any suitable index that might be used to make this conversion. One cheese factory can be justly able to outpay another because of better quality cheese due to better quality milk, superior manufacturing practices for producing quality cheese, better or specialized marketing facilities, more efficient factory arrangements, more systematized operation because of more efficient management and greater use of labor saving devices. Labor and capital investment are a considerable part of the costs of making cheese. The estimated cost of labor per lb. of cheese has increased from 0.59¢ in 1909 to approximately 2.09¢ in 1949. Gross income per dollar of depreciated property should be at least $8.11. An average of $1.00 of working capital should be provided for $8.91 of gross income. The average profit margin per dollar of gross income is 2.5% for the dairy industry. This is low when compared to 5.25% for all manufacturing industries. H. E. Calbert

220. Slash repairs and keep rolling with handy records. O. L. APPLEGATE, Standard Brands, Inc., Pekin, Ill. Operating Eng., **4,** 1: 20, 23. Jan., 1951.

Because of divided responsibility between equipment operators and maintenance personnel, some equipment may be neglected in the maintenance program. To avoid this an efficient program and record system should be effected. All equipment should be designated by code numbers for identification. Next, establish frequency of inspections and maintenance for each piece included in the program and to prepare the schedule. Instructions should be typed on cards or placed in a maintenance control book.

Work orders for the current month's maintenance are prepared from schedule and are distributed to the proper personnel for action. After the inspection is completed, the clerk compiles all labor and material requisitions. After the work order has been approved, it is filed under the code number.

Record forms for a complete maintenance procedure include work-order request, time cards, maintenance record, mechanics daily time ticket, material requisition, equipment transfer, property acquired form and miscellaneous work orders cost sheet. These forms are illustrated in detail. H. L. Mitten, Jr.

221. A plant safety program. H. DALLMAN. Butter, Cheese & Milk Prod. J., **42,** 1: 30, 39–40. Jan., 1951.

Safety must be sold to employees. This can be done through a safety committee consisting of members representing both labor and management. This committee should check into all accidents, ascertain their cause and recommend ways of avoiding them in the future. Common causes of accidents are broken guard rails, unsafe ladders, rough floors, improper stacking of materials, oily or slick floors and improper lighting. Good housekeeping will do much to eliminate accidents. The effectiveness of a safety program can be measured by the decrease in accident rates. H. E. Calbert

222. Pattern for disaster. N. MYRICK. Am. Milk Rev., **12:** 5, 2–4, 6, 8, 54, 55. May, 1950.

The price war which began Oct., 13, 1949, on the New York City milk market is discussed. D. J. Hankinson

223. A city owned milk plant. W. RUDOLPH. Am. Milk. Rev., **12,** 4: 4–6, 72. Apr., 1950.

Jamestown, N. Y. is considering the establishment of a municipal milk plant. Employees of a city-owned milk plant would enjoy the same benefits as other municipal employees. Economic advantages claimed for such a plant include (a) reduction in price of milk to the consumer, (b) elimination of 12 dairy plants now in operation on which options have been received, (c) 1 truck for a given area instead of 2–15 as at present, (d) 20–25% saving because gasoline and some equipment will be tax-free and (e) "free" use of steam from the city light plant.

Milk dealers in Jamestown oppose this plant. They favor free enterprise and claim that their milk prices are not high in view of the high quality milk. To avoid a price war, all but 2 of the dealers have signed options if the municipal plant idea does meet with public approval. D. J. Hankinson

Also see abs. no. 240.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

224. Formation of stereoisomers of beta-carotene in alfalfa. C. R. THOMPSON, E. M. BICKOFF and W. D. MACLAY. Western Reg. Research Lab., Albany, Cal. Ind. Eng. Chem., **43:** 126–129. 1951.

The effect of commercial dehydration of alfalfa at 4 industrial plants showed at what stages the formation of carotene isomers occurred. The effects of heat, light and the addition of antioxidant were evaluated. Increased temperature promoted the formation of *cis* isomers. The amount of *cis* isomers was decreased and the provitamin A potency of a meal increased by irradiation with visible light. Neo-β-carotene B was destroyed more rapidly than the other isomers during storage. The ratio of isomers remaining after storage was only slightly affected by the presence of an antioxidant. B. H. Webb

225. Aspects de l'alimentation de la vache laitière (Aspects of the nutrition of the dairy cow). R. FERRANDO. Lait, **30,** 299–300: 612–622. Nov.–Dec., 1950.

This paper constitutes a discussion and review (without bibliography) in which various phases of ruminant nutrition are considered in the light of the author's work as well as that of others.
S. Patton

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

226. The effect of reducing the quantity of egg yolk in bovine semen diluents. D. L. STEWART, D. R. MELROSE and W. R. WILSON, Ministry of Agr. and Fisheries, Reading. Vet. Record, **62,** 44: 617–618. 1950.

A control diluent of equal parts egg yolk and 3% citrate solution was compared with a trial diluent composed of 1 part of egg yolk and 3 parts of citrate buffer. Refrigerator storage tests of 13 d. showed no significant difference between the control and trial diluents. The conception rate for the fertility trial was estimated on the basis of non-returns to first inseminations in 16 wk., and there was no significant difference in the fertility of the semen with the trial or control diluents. The fertility trial involved approximately 1,800 first inseminations for each diluent.
R. P. Niedermeier

227. A seminal defect associated with sterility of Guernsey bulls. J. L. HANCOCK and D. H. L. ROLLINSON. Vet. Record, **61,** 45: 742–743. 1949.

A morphological abnormality of spermatozoa found in 12 young Guernsey bulls with a breeding history of total sterility is described. Photomicrographs show an absence of intact spermatozoa, with only free heads and tails present; no sample examined had more than 5% intact spermatozoa. This characteristic feature was observed in 73 ejaculates studied from the 12 bulls, including repeated samplings for periods of 1–8 mo. Motility ratings on the semen gave low values, and microscopic examination of diluted semen at the time of collection indicated the free tails and heads already were present, with the free tails having some movement. Motility was maintained for periods of 36–116 hr. when the semen was stored at 4° C. Density values ranged from 100,000 to 900,000 sperms/ml. In stained slides of the separated heads and tails, the heads appear normal with a deep indentation at the point of separation, but most of the tails are abnormal. Pedigree examination of the 12 bulls indicates the abnormality is not of genetic origin nor could any other common environmental factor be found. R. P. Niedermeier

Also see abs. no. 174.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

228. Loose housing for dairy cattle. S. A. WITZEL, Univ. of Wis., Madison. Milk Dealer, **40,** 2: 47–48, 54–56. Nov., 1950.

The following simple rules for planning a loose housing system result from: (a) Bedded area of 60 sq. feet per cow plus necessary pen space for calves, young stock and hospital needs and plus space either at floor level or in a loft above for adequate bedding storage. This area should be graded up above outside ground level but a dirt floor is adequate. (b) Feeding area at least for hay under roof. This paved feeding area with a south or east side opening out onto a wind-protected paved lot is most convenient. Hay should be stored above or alongside the manger. Where hay and silage are fed at the same manger, allow 27–30 in. of feed manger per cow. To save time, silage often is fed out of doors so hay and silage may be fed at the same time and once a day only. In such an arrangement from 1.5–2 ft. of manger per cow is adequate. (c) The paved barn lot providing about 100 ft.^{2} of concrete per cow plus a sodded pasture available in winter and when the ground is firm and dry for the cows to get off the concrete and on warm fall and spring nights for them to rest out in the open, is a "must". Good drainage and good wind protection are essential, while good board or plank fencing is the safest for the cows. The barnyard should be cleaned as required. (d) Most important is the farm milking plant, which includes the milking stalls and milk house. This building must be built to meet all sanitary requirements, the same as it is necessary to operate the rest of the loose housing system so the cows are kept clean.
C. J. Babcock

229. Vacuum cleaner for cattle. W. LIMBERG U. S. Patent 2,539,357. 3 claims. Jan. 23, 1951. Official Gaz. U. S. Pat. Office, **642,** 4: 1191. 1951.

Dirt, dust, loose hairs, etc. are removed from cows by vacuum supplied by a portable vacuum cleaner. Moist, heavy, sticky dirt collects on a removable baffle-like screen, whereas the lighter dust, hairs, etc. are collected on another screen positioned behind the baffle and before the motor-driven fan. R. Whitaker

230. Stock watering fountain. A. F. KLINZING. U. S. Patent 2,539,785. 3 claims. Jan. 30, 1951. Official Gaz. U. S. Pat. Office, **642,** 5: 1403. 1951.

Water is admitted to the bowl of this fountain as long as the animal's nose depresses a lever.
R. Whitaker

231. Method of making milker pails. F. J. J. HENRARD (assignor to Babson Bros. Co.). U. S. Patent 2,538,098. 8 claims. Jan. 16, 1951. Official Gaz. U. S. Pat. Office, **642,** 3: 821. 1951.

A welded stainless steel milker pail of the suspended type is described. R. Whitaker

232. Control device for milking machines. A. G. PERKINS and R. DU QUETTE. U. S. Patent 2,538,652. 17 claims. Jan. 16, 1951. Official Gaz. U. S. Pat. Office, **642,** 3: 964. 1951.

A device for automatically controlling the pulsations of a vacuum-type mechanical milker is described. R. Whitaker

Also see abs. no. 241.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

233. Ice cream freezer. N. E. GADDINI. U. S. Patent 2,541,814. 18 claims. Feb. 13, 1951. Official Gaz. U.S. Pat. Office, **643,** 2: 616. 1951.

A vertical dasher-type batch ice cream freezer is designed to operate within a room or area of frigid air. One motor operates a fan to force the chilled air around the freezing chamber and also operates the dasher and scraper blades.
R. Whitaker

234. New frozen citrus purees from citrus fruits. E. A. BEAVENS, Bureau of Agr. and Ind. Chem., Pasadena, Cal. Ice Cream Rev., **33,** 3: 110, 112, 114. Oct., 1949.

Methods of preparation, packaging and freezing have been developed for the citrus purees which are well adapted for use in the ice cream and other food industries. Sound mature fruit is washed, stemmed, trimmed and crushed. The crushed fruit is reduced to a puree by a mechanically-driven screening device with air incorporation kept at a minimum. The yield of puree is 50–60% of the whole fruit and contains 0.65–0.75% of peel oil. Five parts of puree are mixed with 1 part sugar in a stainless steel tank. The sweetened puree is placed in lacquered or enameled cans of from 1–2.5 gal. capacity. The cans either are hermetically sealed or closed with slip top covers and the contents frozen rapidly and stored at 0 to −10° F. These purees have been held for more than a year without change in flavor, color and with little or no loss of vitamin C.

Although citrus purees have been used successfully in the preparation of both sherbets and ices, better results were obtained when they were used in sherbets than in water ices. A sherbet mix containing 2.5% fat, 2.5% serum solids, 25% sugar and a suitable stabilizer gave good results with citrus purees. In preparation of orange sherbet 15–18 oz. of the 5:1 puree and 1.5 oz. of 50% citric acid solution/gal. of mix proved satisfactory. When making a lemon sherbet it was found desirable to use only 10–14 oz. of the puree and 0.5 oz. of citric acid/gal. of sherbet mix.
W. J. Caulfield

235. Ice cream scoop. B. F. and E. E. LAURENCE. U. S. Patent 2,540,397. 4 claims. Feb. 6, 1951. Official Gaz. U. S. Pat. Office, **643,** 1: 144. 1951.

A bowl-shaped scoop of the customary shape with the ejector operated by a shaft through the center of the bowl is described. R. Whitaker

Also see abs. no. 176, 209.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

236. Picking up milk by tank truck. Anonymous. Am. Milk Rev., **12,** 4: 64, 66. Apr., 1950.

A tank route is operated by a dairy plant in So. Carolina and also by one in Connecticut. The Connecticut dairy has an 8-stop route which has proved satisfactory to both the plant officials and producers. Stainless steel holding tanks are used which contain direct expansion surface cooler and over which the milk is poured directly from the milking machine pail. Within 10 min. the milk is cooled to 38–40° F. The milk is collected in a 1,600-gal. tank truck by a driver who takes butterfat samples daily and a sample for bacteria count each week. The quantity of milk in the farmer's tank is determined by a calibrated metal measuring stick. The producer cleans the holding tank. This system of handling the milk is favored by producers because of the method of weight determination, butterfat sampling, elimination of can handling and the additional space provided when the large can-cooling tanks were replaced. Milk quality has been improved.

The So. Carolina route operates with only slight variations. Cold walled holding tanks were used at each of the 6 stops. 10 gal. of chlorine sterilizing solution are placed in the tanker at the start of each trip and drained out at the first stop. A premium of 12¢/cwt. is paid for milk with a count of under 50,000/cc.
D. J. Hankinson

237. Milk distribution through vending machines. E. THOM, Olsen Publishing Co. Milk Dealer, **40,** 3: 38–39, 72–77. Dec., 1950.

The distribution of milk through vending machines can be more economical than either retail delivery or wholesale distribution through stores. Moreover, the milk dealer receives his money promptly, since the machine business is a cash business. A complete description of the vending machine operation by the City Milk Co., Inc., of 58–64 Maurice Ave., Maspeth, Long Island, in the N. Y. Metropolitan area is given. This firm started with 5 vending machines in 1937 and now covers the entire metropolitan area with several hundred machines in operation. C. J. Babcock

238. The Boston program for quality control of cream. F. E. MOTT, Boston Health Dept. Am. Milk Rev., **12:** 5, 46–49. May, 1950.

The Boston cream market is supplied by licensed producers. Out of state producers are licensed on the basis of government inspection. Each load of cream shipped to Boston has the following tests made by the shipper: (a) microscopic examination, (b) standard plate count at 32° C., (c) acidity, (d) phosphatase and (e) neutralizer. The results of these tests are recorded on a warranty form which is sent immediately, by air mail, to the buyer, who in turn makes the same tests of each load of cream and records his results on the back of the warranty to the Boston Health Dept., where the cream shipment is classified as for bottling, manufacturing or illegal.

The author defines cream of good quality as pasteurized, free from foreign substances including added water, and having a standard plate count at 32° C. of less than 40,000 colonies of bacteria /cc. D. J. Hankinson

239. Some causes for poor cream quality. T. MARCUS, Mass. Dairy Labs., Boston, Mass. Milk Dealer, **40,** 2: 114–116. Nov., 1950; Am. Milk Rev., **12:** 3: 54, 55, 57, 58. Mar., 1950.

This is a discussion of cream quality which establishes the following rules which must be followed in making cream that will have a low count and good keeping quality. (a) Pasteurize cream at 160° F. for 33 min. (b) Cool to below 40° F. and place in a blower chest to cool below 34° F. for storage at that temperature. Bottle cream should be iced after bottling. (c) Sterilize all equipment immediately before using with 180–190° F. water. The simpler the equipment, the better. (d) Steam clean the cream cans not more than 1 hr. before filling. There is no substitute for heat in sterilizing equipment or cans. Good cream after 7-hr. incubation at 90° F. will show a low plate count and be negative for *E. coli.* C. J. Babcock

240. Monopoly in the east. R. W. BARTLETT, U. of Ill. Am. Milk Rev., **12:** 5, 10, 12, 14. May, 1950.

The author has used a question-answer form to present the problems created by 8 of the 35 federally-regulated milk markets. These markets, located in the east, have monopoly Class I price. This has had an unfavorable effect on the producers' market in the west. The 2 alternatives suggested were: (a) each of the 8 federal order markets lower their Class I price or (b) the federal order in each of these 8 markets be cancelled. D. J. Hankinson

Also see abs. no. 190, 211, 212, 213, 215, 223.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

241. Practical observations on milking machine sanitation. J. C. FLAKE. Butter, Cheese & Milk Prod. J., **41,** 12: 26–27, 37–39. Dec., 1950.

Methods for cleaning milking machines that seem to be most satisfactory under experimental tests may not obtain the best results in the hands of the typical dairy farmer. The method that will work for a particular producer is the one he should follow. A joint program of the IAMFS, USPHS and the DIC is aimed at improving and standardizing milking machine construction so that they may be cleaned more easily. A study of frequency of parts found dirty, based on examination of over 1,000 milking machines, revealed the pail head to be most frequent offender. Inflations were second, pail third, long milk tube fourth, and finally, the claw. Sanitarians and fieldmen can do much to help the dairy farmer keep his milking machine clean. Fieldmen or sanitarians must be thoroughly familiar with construction, operation and sanitation of milking machines in order to gain the confidence of the dairy farmer. This will get him to do a good job of cleaning his dairy equipment. A simple and time-saving method of cleaning milking machines consists of flush-washing the machine immediately after each milking. This is followed by storage with dilute lye solution. This method will not clean up a dirty machine but will keep a clean machine in good shape. H. E. Calbert

242. The care of stainless steel dairy equipment. R. E. PARET, Am. Iron and Steel Inst. Am. Milk Rev., **12,** 3: 30, 32. Mar., 1950.

The following cleaning rules were approved by the Technical Committee of the Dairy Industry Supply Assoc. in cooperation with engineers affiliated with fabricators of stainless steel equipment, to prolong the life of all stainless steel dairy equipment: (a) Immediately after using, rinse with cold water until the walls are cool, (b) clean thoroughly with warm water and commercial dairy cleaner, and brush thoroughly, removing any stubborn particles with stainless steel sponge or wool, (c) rinse thoroughly with hot water and allow to dry.

The equipment must be sterilized just before it is to be used. Heat sterilization may be accomplished by filling with water at 170–180° F. for 5 min. or with live steam for 5 min. Do not allow the steam to strike directly on the walls or bottom. Chemical sterilization may be accomplished with a chlorine solution or other germicidal agent. Residual chlorine in the solution after use should not exceed 50 ppm.

Rust, discoloration or pitting may result from: the use of ordinary steel wool; use of water containing Fe, salt or S; allowing chemical sterilizers, alkalies or cleaners to remain on too long; allowing particles of foreign matter to adhere to equipment; leaving on rubber protective items to prevent drying; or allowing dissimilar metal parts to lie on wet surfaces. Care also must be taken not to close the covers of a tank after steaming to create a vacuum, nor to apply air or steam pressure greater than the equipment is designed to withstand. D. J. Hankinson

243. Looking at can washing operations. H. N. NUPSON, Redwood Falls, Minn. Milk Dealer, **40,** 2: 31–135. Nov., 1950.

This is a discussion of milk cans as a cause of off-flavors, high bacterial counts and bad sediment pads of milk and cream delivered to plants.

Can washing methods and points to check to insure proper can washing operations are outlined.
C. J. Babcock

244. Effective and economical cleaning. J. R. PERRY, Nat'l. Dairy Prod. Co., Inc. Am. Milk Rev., **12,** 4: 32, 33, 76–78. Apr., 1950.

This study showed that if plants had proper fundamental facilities, effective cleaning could be accomplished with a great saving in cost of cleaning facilities as well as in maintenance cost.

Rinse water at 115° F. was most effective. This temperature could be attained by use of a hot water generator, preferably with a reliable and sensitive thermostatic control. When water with differing temperatures is desired from 1 hot water generator, tempering valves which mix the hot and cold water may be installed.

Correct water pressure also is important. This varies with different tasks in the plant and may be controlled for various requirements with a pressure-regulating valve, and preferably by a controlled-pressure water station; this consists of a pressure-regulating valve, a strainer, pressure gauge and a master valve.

Except where a large flow of water is needed, the 0.5″ special lightweight hose is most satisfactory. It is best to have a shut-off valve attached to the end of the hose. A "whip-end" must be used between the end of the light-weight hose and the shut-off valve. Attention should be given to use of the proper nozzle, such as the blade nozzle for all close-up rinsing, and the round nozzle asssembly for rinsing at a distance. Should it be necessary to detach shut-off valves from hoses when not in use, a quick detachable coupling may be attached where the whip-end is connected to the lightweight hose.
D. J. Hankinson

245. Special equipment for effective and economical cleaning. J. R. PERRY, Nat'l. Dairy Prod. Co., Inc. Am. Milk Rev., **12:** 5, 28–30, 32, 56, 57. May, 1950.

The author has presented and described in detail the following pieces of equipment: (a) sanitary pipe cleaner, (b) sanitary pipe truck, (c) sanitary pipe cleanerette, (d) sanitary fittings cleaner, (e) sanitary fittings buggy and (f) a combination parts cleaner. D. J. Hankinson

246. The control of microorganism populations. A. L. STOTIER, Wyandotte Chemicals Corp. Milk Dealer, **40,** 2: 41–42, 84–85. Nov., 1950.

The various natural and man-made agencies for control of microorganisms are discussed.
C. J. Babcock

247. The control of microorganism populations. A. L. STOTIER, Wyandotte Chem. Corp. Milk Dealer, **40,** 3: 45–46, 59–60. Dec., 1950.

As of 1950, 4 major classes of germicides are recognized. These are the phenolics, the heavy metals, the chlorine-liberating and the quaternary ammonium germicides. The alkalies may be regarded as a fifth class, but they are usually employed for purposes other than disinfection. Phenol is rarely used outside of the medical field and even here its usefulness is very limited because of corrosive action and toxicity. The heavy salts are far too toxic for general use, and should never be associated with foods or food handling equipment. While chlorine is difficult to handle and presents hazards, several chlorine-containing chemicals have the property of liberating chlorine slowly and safely, so that these chlorine-liberating chemicals offer a practical means of achieving the same results that chlorine gas offers. The hypochlorites and chloramines and their sterilizing action are discussed. C. J. Babcock

248. The control of microorganism populations. A. L. STOTIER, Wyandotte Chem. Corp. Milk Dealer, **40,** 4: 45–46, 74–78. Jan., 1951.

The characteristics of quaternary ammonium germicides are discussed. C. J. Babcock

Also see abs. no. 181.

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