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Contents

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LACTOSE AND ITS UTILIZATION: A REVIEW

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INTRODUCTION

For thousands of years previous to the seventeenth century, milk was considered as having only three components, since it was common practice to remove fat and curd, either separately or together, thus leaving a third substance, whey. Whey, or serum, appears to have been used in considerable quantities by physicians of the time of Hippocrates and Galen and through the middle ages without realization that its effects were due chiefly to one specific component (8).

The first record of the isolation of the "essential salt of serum" was published in 1633 by Bartolettus (3, 11, 15), who in 1619 had written of milk as composed only of fat, serum and curd (2). Ettmüller, in 1688 (5), published improvements on Bartolettus' process of evaporation and included the purification of the crude lactose by recrystallization.

During the eighteenth century, lactose became a commercial commodity (1, 7, 10), its use being principally in medicine in place of the whey formerly used (4, 6, 8, 12, 13). The foundation of our present knowledge of lactose was laid during the nineteenth century and from this basis there has developed over the past fifty years the present structure of understanding of the characteristics and utility of this unique sugar.

By far the greatest proportion of lactose is consumed as a component of milk. The lactose isolated from milk and refined is used almost entirely in foods for infants and invalids and in pharmaceutical preparations as an

excipient. Many other uses, such as in silvering mirrors, for preserving latex and oilcake and for giving a frosty appearance to certain bottled liqueurs, are listed by writers on lactose, but these are of negligible commercial importance. Whey, because it is relatively cheap and contains readily fermentable lactose, is used as a medium for the production of lactic acid and riboflavin and has been advocated for use in the production of ethanol and penicillin.

Lactose, or milk sugar, has been found in the milks of all species of mammals, the approximate range being from 2.0 to 8.5 per cent (14). Cow's milk normally contains about 4.8 per cent. No other sugar is present in any appreciable percentage in milk, and it is probably true that the only natural process in which lactose is formed is that of lactation.

For the calculation of the lactose available annually in unprocessed whey, the data of the year 1940 may be taken as representative. In that year there were produced as a byproduct of cheese manufacture 6 billion pounds of sweet whey, of which one billion pounds was converted to whey powder. The remaining 5 billion pounds contained 225 million pounds of lactose. There were also 2 billion pounds of whey from the manufacture of casein and skim milk cheese, from which should be deducted the 200 million pounds of casein whey used in the production of 5 million pounds of refined lactose. The 80 million pounds of lactose originally present in the milk from which the remaining 1.8 billion pounds of whey was derived had been in part fermented to lactic acid, which reduced its degree of availability except for complete conversion to lactic acid. However, since sweet whey contains 5 per cent of lactose rather than the conservative 4.5 per cent used in these calculations, 300 million pounds of lactose may be considered to have been potentially available in unprocessed whey in 1940. For the war year of 1942, the corresponding quantity of lactose in unprocessed whey was over 400 million pounds.

STRUCTURE

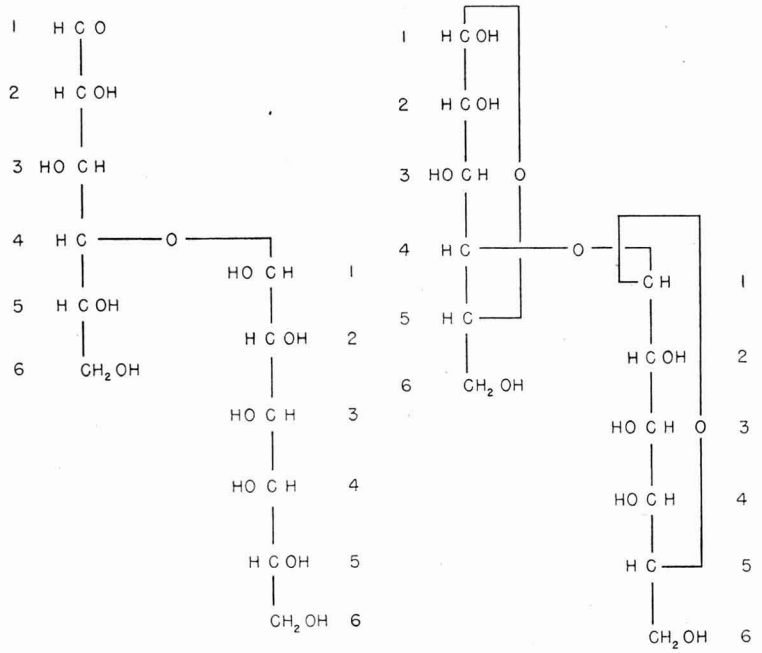
Beliefs regarding the structures of sugars have changed fairly frequently during the past fifty years and it is possible that facts yet undiscovered may change current views of the manner of linkage both within and between monosaccharides. It seems proper only to mention here chronologically the evidence that has led to the structure that is generally accepted for lactose at the present time.

In 1812, when lactose was first hydrolyzed (45), it was supposed that the substance produced was glucose only. The establishment of the proportions of the elements in lactose (44), the discovery that hydrolysis of lactose gives a sugar that is not glucose (19, 39), and the discovery of the aldehydic reducing action of lactose (37) in 1855 and 1856 led to the establishment ten years later (23) of the fact that *two* hexose sugars, galactose and glucose, are the products of hydrolysis of lactose.

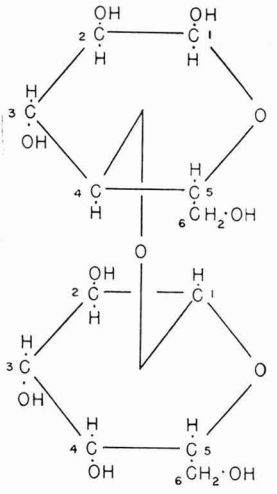
In 1888, Emil Fischer hydrolyzed lactosone, obtaining glucosone and galactose (20), and in the following year he hydrolyzed lactobionic acid, obtaining gluconic acid and galactose (21). Since the reactions producing osones and monobasic acids from aldoses take place at the aldehyde end of the sugar molecule, it was concluded that the aldehydic portion of lactose is the glucose residue, and that the aldehyde group of galactose is not present as such in lactose, but must be the point of union of the galactose to the glucose residue. Hence lactose is a galactosyl glucose. This conclusion has been verified by many similar experiments.

Since one of the products of the hydrolysis of methylated lactose is 2, 3, 6-trimethyglucose (26), the point of union of the glucose residue should be on either the 4 or 5 carbon. The correct choice depends upon whether the lactone ring has a 1–4 (furanose) or a 1–5 (pyranose) linkage. Evidence as to which of these rings is normal for monosaccharides and, furthermore, as to whether the rings in polysaccharides are necessarily the same as those of the monosaccharides formed by hydrolysis has been confusing and even conflicting (17, 27, 28, 30, 32, 33, 35, 36, 46), but the pyranose structure (24) is generally accepted at present for lactose and for both the glucose and galactose components. That the glucose union is at carbon 4 has been shown by other means as well. Whether lactose, and consequently the glucose residue in lactose, has the alpha or beta configuration is easily decided, since the designation alpha is arbitrarily assigned to the form having the greater rotation in the dextro direction. That the configuration of the galactose residue in lactose is of the beta form has been shown by Fischer (22), who found that an enzyme that hydrolyzed lactose would hydrolyze a β-methylgalactoside, but not an α-methylgalactoside, and that an enzyme that hydrolyzed an α-galactoside, but not a β-galactoside would not hydrolyze lactose. The arrangement of the H and OH groups on the other asymmetric carbon atoms of the hexose residues is a matter on which there is general agreement and will not be discussed here, except to point out that the structural difference between glucose and galactose is in relative arrangement of the H and OH groups on carbon atom 4.

The accompanying formulas are in agreement with accepted facts. I is a formula representing in one plane the very small percentage of lactose believed to be present in lactose solutions in the form of a chain, with a functional aldehyde group at carbon 1 of the glucose residue. IIa shows in one plane the 1–5, or pyranose rings, of the predominant, lactonic form (27). IIb is a formula of the Haworth type (30), indicating the relative positions of component groups in three-dimensional space. Atoms placed above the carbon atoms are to be considered as on one side of the plane of the ring, those below on the other, the rings themselves being in different planes. A recent modification (18) of the Haworth scheme of representation is shown as IIc. In this scheme the O atoms of the lactone rings are placed at the top,



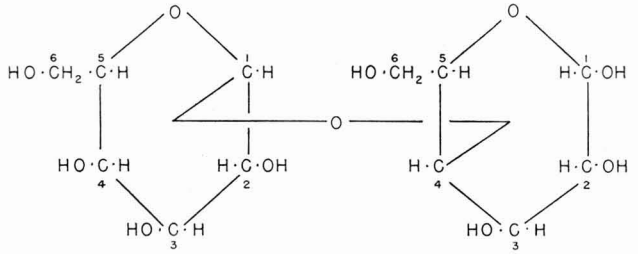
I IIa



IIb

atoms at the right of carbon atoms are considered to be below the plane of the ring, those at the left above. In comparing these formulas, it should be noted that the numbering system is the same in all four, and that IIa, IIb, and IIc differ only in the method of representation. All four formulas are supposed to represent α-lactose; interchange of the positions of the H and OH on carbon atom 1 of the glucose residue or the corresponding change in the position of the lactone ring of the glucose residue converts the formula to that of β-lactose.

An anhydrous modification (31) containing five moles of α-lactose to three moles of β-lactose has been prepared by crystallization from methanol. Epilactose has been prepared from lactose (25) and differs from it in the reversal of the positions of H and OH on carbon atom 2 of the glucose residue, being therefore a galactosyl mannose. Neolactose (34) differs from lactose in that the H and OH groups on both carbon atoms 2 and 3 of the glucose residue are reversed in position. It is, therefore, a galactosyl altrose. Lactulose (38) is a galactosyl fructose.



IIc

Gynolactose, a levorotatory galactosylglucose, and allolactose, a dextrorotatory galactosylglucose, have been isolated in small quantities from human milk (41, 43). Allolactose is believed to be 6-β-d-galactosyl-d-glucose (29, 42), presumably differing in structure from lactose only in that the galactose residue is linked to carbon 6 of the glucose residue rather than to carbon 4.

PHYSICAL FORMS AND EQUILIBRIA

Mutarotation is a phenomenon characteristic of all natural reducing sugars in water solution and in such instances is attributable to changes in the proportion of alpha and beta forms. The mutarotation of lactose solutions was first noted by Erdmann in 1855 (48). Several relevant observations were reported previous to 1900 (47, 49, 59, 60, 61, 63), but the most extensive investigations of mutarotation of lactose solutions and of equilibria among the forms of lactose, both in solution and in the solid state, have been carried out more recently by Hudson (52, 53, 54), by Gillis (50, 62), and by Parisi (57).

The ordinary lactose of commerce has the composition expressed empirically by the formula $C_{12}H_{22}O_{11} \cdot H_2O$. It has the specific optical rotation in water solution $[\alpha]_{20^\circ}^{D} = +89.4^\circ$, a melting point of 201.6° C., and is a monohydrate of 4-β-d-galactosyl-α-d-glucose, being designated more briefly as α-lactose hydrate.

When α-lactose hydrate is dissolved in water, its specific rotation changes gradually from +89.4° to +55.5° at ordinary temperatures, the rate of change being a function of the temperature, of the concentration of H and OH ions in the solution and of the concentration of certain other solutes.

If an aqueous lactose solution, however prepared, is concentrated and crystallization is caused to take place at a temperature below 93.5° C. and at a moderate rate, the crystals formed are α-lactose hydrate. If, however, the temperature during crystallization is above 93.5° C., the crystals have the composition $C_{12}H_{22}O_{11}$, a specific rotation in water solution $[\alpha]_{20^\circ}^{D} = +35.0$ and a melting point of 252.2° C. This form is 4-β-d-galactosyl-β-d-glucose and is usually designated β-lactose anhydride or, more simply, β-lactose.

When β-lactose is dissolved in water, its specific rotation changes gradually from +35.0° to +55.5°, the solution ultimately becoming identical in all respects to one prepared from α-lactose hydrate and water in corresponding proportions. The equilibrium rotation of +55.5° is attained practically instantaneously in lactose solutions at temperatures above 70° C., whether the lactose dissolved was alpha or beta.

If α-lactose hydrate is dehydrated by heating at a temperature below 93.5° C., preferably under reduced pressure and not below 65° C., a variety having the composition $C_{12}H_{22}O_{11}$ is formed. Its melting point is 222.8° C. When it is dissolved in water, the solution manifests the same rotation and mutarotation as one containing the corresponding quantity of the hydrate, and therefore this compound is designated α-lactose anhydride. This anhydride is stable in dry air, but in the presence of moisture it changes to β-lactose anhydride at temperatures above 93.5° C., to α-lactose hydrate at temperatures below 93.5° C.

A solution of lactose in a state of rotational constancy, or equilibrium, at 25° C. has 62.25 per cent of its lactose in the β form and 37.75 per cent in the α form. The equilibrium constant is therefore 1.65 at this temperature. This ratio is unaffected by changes in pH value of the solution, but is altered by changes in temperature, being 1.62 at 20° C. and 1.81 at 49° C. according to Kendrew *et al.* (55). According to Gillis (50) K decreases with rise in temperature, being 1.65 at 0° C. and 1.33 at 100° C.

If an equilibrated solution of lactose is practically instantaneously deprived of its water, as may be done by a spray or drum dryer, the resulting solid is an amorphous glass containing the β and α forms in the equilibrium

ratio. Drying more slowly above 93.5° C. increases the ratio of β to α and can even result in practically pure β-lactose.

The velocity constant of approach to equilibrium in solution, k_1 (for α to β) + k_2 (for β to α), is strongly affected by changes in temperature and in the concentrations of components of the solution, particularly H and OH ions. Hudson found the relative percentages of α transformed in one hour to be 3.4 at 0° C., 17.5 at 15° C., and 51.1 at 25° C. At 70° the change is practically instantaneous. These rates should be remembered when evaluating claims of differences in properties of α- and β-lactose determined by comparisons made on solutions of the two forms, particularly when such solutions have stood for some time at an elevated temperature.

An equation has been derived by Parisi (57) for the velocity constant of the β to α change, $k_2 = ax^{pH} + by^{pOH}$, in which a and b are coefficients depending on temperature and x and y are values of the rate of change as a function of OH-ion and H-ion concentrations, respectively. k_2 is at a minimum at approximately pH 5.0 and the effects of the concentrations of H and OH ions are equal at approximately pH 7.0. Since the effect of OH-ion concentration is much greater than that of H-ion concentration, k_2 increases more rapidly with increase of pH above 5.0 than with decrease of pH below 5.0. Since the ratio between k_2 and k_1 is very nearly an absolute constant, an analogous equation may be written for k_1. In applying these equations, it is necessary, in converting values of pOH to equivalent values of pH, to take into account the variation of the value of K_w with temperature (51).

Values of k_1 and k_2 may be calculated from experimentally determined values of $k_1 + k_2$, since k_1/k_2 is determinable from the ratio of the components of an equilibrated solution.

An empirical equation has been formulated by Herrington (51) for calculating the mutarotational constant in terms of pH values: $\log (k_1 + k_2) = 0.00415\ (pH - 4.45)^4 - 0.34$. This equation is applicable only at 25° C. and in the absence of catalytic substances other than H and OH ions.

The presence of lactate or of acetate in normal concentrations at pH values of 4.0 to 5.0 increases the value of $k_1 + k_2$ from 4 to 8 times. Presumably other weak acids have similar catalytic effects.

Several conclusions may be drawn from the above facts. It is not proper to consider the hydrate of lactose as neither α nor β; but, since the hydrate and α-lactose anhydride have the same molecular rotation when freshly dissolved and show the same mutarotational changes, they are properly classed together as of the α variety, in contrast to the β-anhydride, which has a different molecular rotation. A saturated aqueous solution of lactose at 93.5° C. is saturated with respect to both the α and β forms. This does not mean that the two forms are present in equal quantities; the equilibrium persists. A saturated solution at temperatures above 93.5° C. is saturated with respect to β, but not with respect to α. A solution saturated at temperatures below

93.5° C. and in α–β equilibrium is saturated with respect to α, but not to β. Though β-lactose hydrate has not been prepared, it can be predicted that it would be more highly soluble than the β-anhydride and, in the solid form, would be unstable, changing to the β-anhydride above 93.5° and to the α-hydrate below 93.5° C. The transition point for solid α-lactose hydrate ⇄ solid β-lactose anhydride is 93.5° C. A transition point for solid α-lactose anhydride ⇄ solid β-lactose anhydride cannot exist below the melting points of the two forms.

Two representations have been offered for the equilibria in lactose solutions (50, 54):

1. $\alpha\text{-form} + H_2O \rightleftarrows \underset{\text{(fast)}}{\text{hydrate}} \rightleftarrows \underset{\text{(slow)}}{H_2O} + \beta\text{-form}$

2.
$$\begin{array}{ccc} \alpha\text{-anhydride } (+H_2O) & \rightleftarrows & \alpha\text{-hydrate} \\ \Updownarrow & & \Updownarrow \\ \beta\text{-anhydride } (+H_2O) & \rightleftarrows & \beta\text{-hydrate} \end{array}$$

Objections have been raised to the first on the grounds that it postulates relative speeds of equilibration that must be considered reversed in magnitude for the analogous equilibria for maltose, and that, since the hydrate is assumed to have a terminal group —$CH(OH)_2$ and therefore to be neither α nor β, it contradicts the existence of the lactonic structure for the hydrate and the asymmetry of the terminal carbon atom. Both representations are open to objection in that the assumption is made that lactose exists in solution in both the anhydrous and monohydrated forms, three forms in the first, four in the second, and, by implication, in no other degree of hydration. Freezing-point measurements have indicated that lactose in solution may be hydrated with an average of 3 to 6 molecules of water per molecule of lactose, the precise number depending on the concentration of the solution (66). In any case, the assumption that the degree of hydration of solid forms is an index to the degrees of hydration of forms in solution lacks adequate support. On the basis of current knowledge, inclusion in the representation of equilibrium in solutions of lactose of details involving hydration seems unjustifiable.

SOLUBILITY AND CRYSTALLIZATION

The solubility of lactose in water is shown in table 1, which contains experimental and calculated values of Hudson (72), of Gillis (68) and of Leighton and Peter (74). The value for the initial solubility of β-lactose at 0° C. is the result of a direct determination, that at 100° was calculated from the final solubility of lactose at 100° on the assumption that the equilibrium constant K equals 1.50 and that the solubility of one form of lactose is unaffected by the presence of the other form in the solution. The values for initial solubility of α-lactose above 25° C. were calculated on the basis of the same assumptions. Gillis claims to have shown that the solubility of either

form is unaffected by the presence of the other and, by theoretical calculation and by experiment, to have proved that the equilibrium constant K decreases with rise in temperature. Kendrew and Moelwyn-Hughes (55), on the other hand, have both calculated and determined values of K that increase with rise in temperature. Until one can be sure as to the effect of temperature on the value of the equilibrium constant, the magnitude of mutual solubility effects will be uncertain.

The values of final solubility are the same regardless of the form initially dissolved and refer to solutions in α–β equilibrium and saturated with the α form if below 93.5° C., with the β form if above 93.5° C.

TABLE 1

Solubilities of lactose

Temperature	Initial solubility		Final solubility	Supersolubility
	α	β		
°C.	*g. per 100 g. water*	*g. per 100 g. water*	*g. per 100 g. water*	*g. per 100 g. water*
0	5.0	45.1	11.9	25
15.0	7.1	.....	16.9	38
25.0	8.6	.....	21.6	50
39.0	12.6	.....	31.5	74
49.0	17.8	.....	42.4	.....
59.1	.....	.....	59.1	.....
63.9	.....	.....	64.2	.....
64.0	26.2	.....	65.8	.....
73.5	.....	.....	84.5	.....
74.0	34.4	.....	86.2	.....
79.1	.....	.....	98.4	.....
87.2	.....	.....	122.5	.....
88.2	.....	.....	127.3	.....
89.0	55.7	.....	139.2	.....
100.0	.....	94.7	157.6	.....
107.0	.....	.....	177.0	.....
121.5	.....	.....	227.0	.....
133.6	.....	.....	273.0	.....
138.8	.....	.....	306.0	.....

The supersolubility values for lactose are definite and readily reproducible. In general the supersolubility at any temperature is equal to the saturation value at a temperature 30° C. higher. The practical significance of supersolubility lies in the fact that solutions supersaturated to a degree not exceeding the supersolubility value will not crystallize, even if the solution is agitated, unless lactose crystals or particles of some isomorphous substance are introduced. Even then, a general crystallization may not occur, but only growth of the nuclei ensue. Herrington (70) has demonstrated that solutions saturated with lactose at approximately 50° C. may be cooled to 0° C. without spontaneous crystallization taking place, provided the solutions are not agitated.

It should be borne in mind that solutions of lactose supersaturated to more than a slight degree may be supersaturated to both α- and β-lactose. From such solutions, either form may be made to crystallize initially by seeding with the desired variety. If the form initially separating is the one more soluble at the temperature of the solution, a subsequent seeding with the less soluble form will cause the less soluble form to crystallize and the more soluble form to redissolve until both solubility and α–β equilibria are established in the solution.

In the crystallization of lactose at ordinary temperatures from a solution seeded with the α-hydrate, two factors are involved—the degree of supersaturation and the rate of change of β to α tending to maintain equilibrium. The rate of crystallization has been studied at temperatures in the range of $-5°$ C. to $+30°$ C. (77) and it has been found that for the first two and one-half hours, the rate of crystallization is greater at 30° C. than at 25° C. or at any lower temperature. The quantity of lactose separated is greater at 30° C. than at any lower temperature until about seven hours have elapsed. The most efficient method of crystallizing lactose should be to cool the solution to 30° C., to seed it and stir it at 30° for about 3 hours, then to cool it to near 20° C. and to hold it at that temperature for 3 or 4 hours or until a convenient time to filter. There is a definite disadvantage in crystallizing at temperatures below 20° C.

In a nearly saturated sucrose solution the solubility of lactose is about one-half what it is in pure water (75). Lactose in solution is about one-sixth as sweet as sucrose (67), but the solid sugar seems even less sweet because of its comparatively low solubility and the hardness of its crystals. Solid lactose produces on the tongue a sensation similar to that produced by sand; in fact, the so-called "sandiness" of ice cream is caused by the presence of lactose crystals.

Lactose is insoluble in 95 per cent ethyl alcohol, in methyl alcohol, and in ethyl ether. Pyridine saturated with lactose at 25° C. contains 2.18 per cent of the sugar (71). Lactose may be dissolved in warm acetic acid, either dilute or glacial, from which it separates unchanged on cooling (76).

OTHER PHYSICO-CHEMICAL DATA

Lactose acts as a weak polybasic acid, binding two moles NaOH per mole of sugar and possibly more. Values for the apparent acid dissociation constants according to several investigators are: the first constant, 1.05×10^{-12}, 0.76×10^{-12}, 1.20×10^{-12}; the second, 3.6×10^{-14}, 3.0×10^{-14}, 3.63×10^{-14}; the third, 1.7×10^{-14}; the fourth, 1.6×10^{-14} (84, 93, 96).

The coefficient of cubical expansion of lactose is 0.00911 per degree between 0° C. and 100° C. (87). The specific gravity of the α-hydrate is 1.5453 (81), that of the β-anhydride 1.59. The specific gravity of lactose solutions is not a straight-line function of concentration, being affected by a contrac-

tion which has a maximum value of 0.593 ml. per 100 grams of solution at 20° C. at a concentration of 54.03 per cent. Such a solution contains 18 moles H_2O per mole $C_{12}H_{22}O_{11}$.

The refractive indices of the tomahawk-shaped crystals of α-hydrate are given as $\alpha = 1.517$, $\beta = 1.542$ and $\gamma = 1.550$ (89), and as $\alpha = 1.517$, $\beta = 1.553$ and $\gamma = 1.555$ (97). The refractive indices of crystals of β-lactose are given as $\alpha = 1.542$, $\beta = 1.572$ and $\gamma = 1.585$ (97). The ratios of the axes of crystals of α-hydrate are given as a : b : c = 1 : 0.6215 : 0.2193 (92) and as a : b : c = 1 : 0.3677 : 0.2143 (95). The ratios for the axes of crystals of β-lactose are given as a : b : c = 0.817 : 1 : 0.377 (97). The values mentioned are for crystals of the forms usually seen. However, the crystal habit of each variety of lactose is influenced greatly by conditions under which crystallization takes place. The different types of lactose crystals are discussed in detail by Hunziker and Nissen (86) and by Herrington (83).

The specific heat of the hydrate is 0.299, that of the β form 0.2895; the molecular heat of the solid hydrate is 107.6 cal., that of the solid β form 99.0 cal.; the apparent heat of either form when it is dissolved in water is 147 cal. (90). Other thermochemical data are given in table 2. Extensive

TABLE 2

Thermochemical data on lactose. 20° C.

Heat of	α-hydrate		β-anhydride		α-anhydride	Ref.
	cal. per gram	*Cal. per mole*	*cal. per gram*	*Cal. per mole*	*cal. per gram*	
Combustion	3945.0	1420.0	4162.0	1423.0	.....	91
"	3736.8	1345.2	3951.5	1351.4	.....	94
"	3761.6	1354.7	3932.7	1345.5	.....	79
"	3948.0	1421.0	.....	.....	.....	88
Formation from elements	.....	610.8	.....	535.6	.....	94
" " "	.....	593.5	.....	534.4	.....	79
Solution, initial	−12.0	.....	−2.3	.....	+7.3	85
" final	−11.4	.....	−2.7	.....	+7.9	85
Transition to β	+1.0	.....	.....	.....	+1.0	85
Transition to equilibrium mixture	.....	.....	.....	.....	+1.3	85

data on heat capacity and entropy of lactose will be found in papers by Anderson and Stegeman (78) and Furtsch and Stegeman (82).

MANUFACTURE OF LACTOSE

General considerations. The refined lactose of commerce is a white, odorless powder consisting of crystals that have been pulverized sufficiently to pass a No. 100 screen. It is at least 99.7 per cent pure as determined by the polariscope and contains not more than 0.050 per cent ash, not more than 0.020 per cent nitrogen and not more than 0.020 per cent fat. It complies with the U.S.P. heavy metals test and its solution in water is clear, colorless,

odorless and neutral to litmus paper. More stringent standards are required for bacteriological and other special uses.

For use in modifying milk or as a substrate for fermentation processes, lactose containing comparatively large percentages of the other components of whey is not ordinarily objectionable, and in some instances the presence of these substances is desirable. However, lactose containing excessive quantities of nitrogen compounds develops unpleasant odors if stored for any considerable time under ordinary conditions of humidity and temperature, and this fact should be borne in mind when the cruder grades of lactose are being produced for other than immediate use.

The whey remaining as a byproduct from the manufacture of casein by precipitation with hydrochloric acid is the source of nearly all the lactose produced in the United States. The whey from casein precipitated by means of sulfuric acid is objectionable because of the difficulty of removing slightly soluble sulfates that impart cloudiness to lactose solutions. The whey from casein precipitated by the lactic acid produced by bacterial fermentation of part of the lactose in the milk is not used because of the relatively low percentage of lactose left in the whey. Lactose can be made successfully from sweet whey derived from the manufacture of cheese or rennet casein, the processes differing slightly from that employed when casein whey is used.

Whey contains on the average 5 per cent lactose, a small fraction of a per cent of fat and 1.6 per cent of protein plus ash. In casein whey there is usually about 0.85 per cent protein and 0.75 per cent ash, in rennet-cheese whey 1.10 per cent protein and 0.50 per cent ash. The difference in the percentages of ash is attributable mainly to the calcium phosphate which is present in casein whey in appreciable quantities, but in rennet whey in practically negligible quantities. Rennet whey contains a small percentage of acid-precipitable protein.

If casein whey is concentrated there is obtained a viscous, sirupy mass from which lactose will not crystallize (104). If the whey is adjusted to neutrality from its initial pH value of between 4.0 and 5.0 and then concentrated without filtration, crystallization is still difficult. In order to obtain efficient crystallization of lactose from casein whey, it is necessary to remove the neutralization precipitate, which consists largely of the calcium phosphate mentioned above, and it is desirable to remove the heat-coagulable protein. Rennet whey when adjusted to neutrality forms no neutralization precipitate.

The facts given above are necessary to an understanding of some of the procedures employed in obtaining crude lactose from whey.

Manufacture of crude lactose. Casein whey is heated in iron tanks with live steam to the boiling temperature (117, 121). During the heating, milk of lime is added gradually until the pH value of the whey is 6.2, or the titrat-

able acidity is about 0.05 per cent, expressed as lactic acid. Excess of lime should be avoided. It is not necessary to boil the whey, since the coagulation of the heat-precipitable protein will be complete at slightly below the boiling point. The batch is allowed to stand for a few minutes to allow the coagulum of protein and calcium phosphate to separate from the clear liquid.

The clear whey is drawn to a storage vat that feeds to a double-effect vertical evaporator, the sludge being held back for filtration later. Evaporation of the water is continued until the whey has a concentration of 20° Baumé, or 30 per cent lactose. Foaming during evaporation may be minimized by having the evaporating pan clean at the start and by adding foam-reducing oils.

The thin sirup from the evaporator is filtered through cloth in a filter press and is followed by the sludge from the coagulation vat, the press thus being cleaned of adhering sirup. The wash whey may be combined with the filtered sirup, but preferably is run to the storage vat for clarified whey.

The thin sirup is next concentrated further in a single-effect vacuum pan, in which it is brought finally to a concentration of about 40° Bé., the exact concentration being determined by experienced operators by observing the degree of crystallization, or "graining," that has taken place. Toward the end of the graining process, some operators add hydrochloric acid, principally to diminish foaming, but also to prevent darkening of the sugar and precipitation of salts.

The hot mass is dropped from the pan into crystallizing vats, in which crystallization may be allowed to continue for several days. It is usually considered preferable to employ crystallizers equipped with slow-speed agitators and jackets in which cold water can be circulated. In these, the temperature of the crystallizing mass can be brought in a short time to about 30° C .and later still lower. By controlling the amount of agitation and the rate of cooling, it is possible to obtain uniform crystals of any desired size.

When crystallization is complete, the soft, wet mass is run to a sugar centrifuge, where the crystals are freed from mother liquor and washed with cold water. The washings are run to the vat for clarified whey and the mother liquor may be concentrated to obtain a second crop of crystals. Because of increased viscosity of this second sirup due to accumulation of salts and other substances, it is somewhat difficult to separate the second crop of crystals. The second mother liquor is discarded.

The wet, crude lactose should either be refined promptly or dried in a tunnel dryer to avoid spoilage.

The first crop should comprise about 70 per cent of the sugar present in the whey and contain between 85 and 90 per cent lactose. It will be slightly yellowish in color.

If so desired, cheese whey may be used by the procedure described for casein whey, provided the whey is evaporated to a thin sirup of about 18° Bé. concentration before, rather than after, liming and heating.

If a sweet whey, such as that from the making of Swiss cheese, is used, the costly operations of boiling and preliminary evaporation of the whey and the filtration of the thin sirup and sludge may be avoided (102, 103, 116). The whey is neutralized to a pH value of about 7.0, or a titratable acidity, expressed as lactic acid, of 0.04 per cent, evaporated under vacuum to a concentration of 32° Bé. (read at 50° C.), or 62 per cent total solids, and the lactose grained and crystallized. The filtrate from the centrifugal separation of the sugar may be dried, preferably in a spray dryer, to produce a whey powder relatively low in proportion of lactose and high in soluble protein, milk salts and riboflavin. This product is highly desirable for modifying milk and for general food use.

The procedure for obtaining crude lactose may be modified in such a way that a sugar suitable for some technical uses may be made by only one crystallization (127). Such a lactose contains very little protein and forms in water a clear but slightly yellowish solution that does not foam appreciably on boiling. After the hot, limed whey has been separated from the coagulum of protein and calcium phosphate, its temperature is regulated to the range of 56° to 58° C. and one part of trypsin is added for each 10,000 parts of whey. The action of the enzyme is allowed to proceed for about an hour, the temperature being held between 56 and 58° C. At the end of the holding period, the protein will be broken down into more soluble units which will not contaminate the sugar when it is crystallized later. A sample of the treated whey should not show precipitation when trichloro-acetic acid is added. The whey is evaporated to 20° Bé. and the sirup decolorized, about one pound of carbon being required for each 1000 pounds of sugar in the sirup. It is then filtered, evaporated to 40° Bé., and the sugar grained, crystallized, centrifuged, washed and dried as previously described.

Lactose has also been made as a byproduct of a process conducted primarily for the purpose of reducing the lactose content of skim milk that is to be used in the manufacture of ice cream (113, 126). This procedure is based on research of Leighton and Leviton (112), which demonstrated that the addition of cane sugar to skim milk before condensing lessens the viscosity of the concentrated milk to such a degree that crystallization of lactose takes place readily and the separation of the crystals by mechanical means is easily accomplished.

The procedure recommended is the following: 5.9 pounds of cane sugar is added to each 100 pounds of skim milk, the mixture is forewarmed to 63° C. for 10 minutes, concentrated under vacuum to 70 per cent total solids, transferred to a crystallizing vat, cooled to 25° C., held over night and the crystallized lactose separated by means of a centrifuge or filter press. About 65 per cent of the lactose in the skim milk is removed by this procedure. The crystals are coated with protein and require a refining treatment.

A process of extracting lactose from spray-dried whey has been devised by Leviton (114, 115). The dried whey should be freshly made or should have been stored in a cool, dry place, since the success of this procedure depends on the lactose being in the amorphous state in the powder. The dried whey is stirred rapidly into 15 times its weight of 76 per cent (by volume) ethanol and, after three minutes, the insoluble protein is removed by filtering through a filter press. The filtrate is acidified to a reaction of about pH 3.6 and allowed to crystallize over night. The lactose is removed by means of a centrifuge. Eighty per cent of the lactose in the first filtrate, or 75 per cent of that in the dried whey, is obtained as a white sugar of a high degree of purity. If 95 per cent ethanol is used, the yield is somewhat less and the sugar is in the form of the equilibrium mixture.

Several other methods of separation of lactose have been advocated, such as the drying of whey, followed by fractional extraction with water (109, 111); the freezing out of water from whey below 0° C., followed by crystallization of the lactose at 0° C. (106) or extraction with alcohol (108); and the drying of clarified whey (125). Many patents have been issued on special methods of clarification of whey.

Refining of lactose. For refining (117, 122), the crude sugar is dissolved with the aid of live steam in sufficient water to give a concentration of 20° Bé. To this sirup are added one-fourth pound of a filter aid and one pound of decolorizing paste per 100 pounds of crude sugar in the batch. The decolorizing paste consists of four parts decolorizing carbon, one part hydrochloric acid and enough water to make the paste easy to handle. The batch is heated to boiling and sufficient hydrochloric acid added to produce a titratable acidity of 0.09 per cent, expressed as lactic acid. It is desirable at this point to allow the batch to stand over night in order that the decolorization process shall reach its maximum effectiveness. The following morning the solution is reheated to near the boiling point and sufficient milk of lime cautiously added to reduce the acidity to 0.05 per cent, or to give a pH value of between 5.4 and 5.8. An experienced operator can judge the proper reaction by the degree of flocculation. The solution is then boiled vigorously for a few minutes and allowed to stand until the flocculated mixture of carbon, protein and insoluble salts has settled. It is then filtered, first through cloth in a filter press and again through a sheet of fine-mesh, rag paper supported between two perforated copper disks. If the filtered sirup is cloudy, insufficient lime was used; if the filtration is slow or the solution is colored, too much lime was used.

The filtered solution is acidified with hydrochloric acid to ensure that the salts and protein still present will remain in solution, then evaporated to 40° Bé., and the sugar grained, crystallized, centrifuged and washed with cold water as in the process for crude sugar. The mother liquor is run to the vats for storage of 20° Bé. sirup for crude sugar production. The wash waters are used for dissolving crude sugar for refining.

The wet lactose, after being tested to make sure that it is of the desired purity, is dried in a tunnel or a rotating drum at a temperature of approximately 80° C. From two to three hours are required. The air circulated to the dryer should be filtered through oil-treated glass wool to remove any dust particles that may be present.

The dried sugar is transferred to covered hoppers from which it goes to a pulverizer, where it is ground until practically all will pass a No. 100 screen. The lactose is packed in barrels having two liners, the one next to the wood being of waterproof paper, the one next to the sugar being of fine, unbleached muslin.

The yield of refined lactose is ordinarily expected to be not less than 50 per cent of the sugar present in the whey, but skillful operation of the refining steps will increase the overall yield to 60 per cent.

Manufacture of beta lactose. Because beta lactose is initially more soluble than alpha lactose, there has developed a demand for beta lactose that has been met by the devising of several practical processes for the conversion of alpha lactose partially or entirely to the beta form.

Drying lactose solutions by the spray process produces a mixture of the alpha and beta forms in approximately the equilibrium ratio (105). This mixture is more soluble than alpha lactose initially, but less soluble than the mixture of the two forms in the ratio of their individual solubilities. It has a high rate of solution because of its fine amorphous condition, but is hygroscopic and has poor wetting properties.

A sugar containing between 90 and 99 per cent of the beta form may be made by drying lactose solutions on a drum dryer. The product is somewhat less amorphous in appearance than spray-dried lactose, is much less hygroscopic and has relatively good wetting properties. Bell (105) recommends drying a preheated 80 per cent solution of lactose on an atmospheric drum dryer with a steam pressure of 65 to 75 pounds and a drum speed of 5 to 7 r.p.m. Under these conditions lactose containing over 90 per cent of beta sugar has been produced. The critical factors of speed and temperature would probably have to be determined experimentally for any specific drying unit. Increasing the speed of drying beyond a certain rate decreases the proportion of beta lactose in the dried product.

The process of Supplee and Flanigan (123) consists of drying a lactose solution in the form of a film on a heated surface at a temperature above 100° C. and removing the film from the surface while it is still a paste containing at least 2 per cent water. The lactose crystallizes in the beta form and the heat remaining in the paste completes the drying.

Sharp's earlier process (118) consisted of adding alpha lactose to a saturated solution maintained at a temperature above 93.5° C. and removing an equivalent quantity of lactose in the beta form. Since a solution super-

saturated with lactose above 93.5° C. is more highly supersaturated with respect to the beta form than to the alpha and since equilibrium between the two forms in solution is established very rapidly at that temperature, the beta form crystallizes readily and, at saturation (undersaturation with respect to alpha), alpha can dissolve and will reappear as crystals of beta. By filtration in a heated centrifuge, a sugar containing a high percentage of beta lactose is obtained.

More recently, Sharp and Hand (119) have developed a procedure whereby dry alpha lactose hydrate is heated in a closed container to 120°–130° C. In the presence of the water vapor formed, a solution of alpha lactose forms on the surface of the crystals and beta lactose crystallizes from this solution. When the conversion is complete, or at a desired stage short of completeness, the water vapor is allowed to escape.

The method of Verschuur (110, 124) has apparently not been tried on a large scale, but is of interest because of the claim made that the beta lactose contains no alpha and because of the unusual procedure employed. Pyridine is added to a boiling solution of lactose in water. Pyridine and water are distilled off and then more pyridine is added and the distillation continued. Beta lactose crystallizes from the solution as the proportion of water present becomes small. The crystals are washed with boiling pyridine and finally with hot ethanol and dried.

HYDROLYTIC PRODUCTS

Lactose may be hydrolyzed by lactase from the wall of the small intestine of mammals (131, 133, 134, 135, 137, 139), by emulsin from almonds (134), by lactase secreted by lactose-fermenting yeasts (128, 129, 130, 132, 142, 143) and by dilute solutions of strong acids (136, 138, 140, 141). Glucose and galactose in equal quantities are the immediate products of the hydrolysis. The enzymatic hydrolysis of lactose has not been developed commercially, largely because of the difficulty of obtaining an adequate supply of enzyme.

The rates of hydrolysis of the different forms of lactose have been found to be the same (141). The half-period of hydrolysis of lactose in 3 per cent solution in 0.05 molar hydrochloric acid at 98° C. is 125 minutes; in 18 per cent solution, 94 minutes (136). A 10 per cent solution containing only sufficient hydrochloric acid to give a pH value of 1.2 to 1.3 is practically completely hydrolyzed at 150° C. in an hour. (138). A sirup made under these conditions, concentrated and neutralized has a pleasing flavor and no objectionable saltiness. If concentrations of lactose greater than 10 per cent are hydrolyzed by this procedure, other products, some of which have a bitter taste, are produced at the expense of part of the hexoses. It is possible to remove the bitter substance by treatment with carbon. The other products are desirable in that they largely prevent crystallization of the sirup. The most satisfactory sirups have been made by the hydrolysis of 30 per cent lactose solutions.

PYROLYTIC PRODUCTS

It is a common observation that milk on heating gradually becomes brown, and it is known that lactose is involved in this color formation (145, 157). Ramsey *et al.* (154) claim that caramelization plays no part in the discoloration of dairy products, but that lactose-casein compounds are formed. Kass and Palmer (150) interpret the change as the caramelization of lactose under the influence of casein followed by the adsorption of the lactocaramel on the casein. It is clear that casein promotes the color formation, that it is accelerated in the presence of phosphates and that boric acid and sodium bisulfite, by forming compounds with lactose, hinder the development of color (152, 155). Lactose is the source of the acid, presumably largely formic, that is formed in heated milk (144, 156).

Pyrocatechol has been found in the products of heating a lactose solution to 280° C. under pressure (148). Heating lactose in a solution of sodium hydroxide has yielded a considerable quantity of lactic acid and smaller quantities of formic acid and pyrocatechol (149). Succinic acid has been identified among the products of fusion of lactose with potassium hydroxide (147).

Lactose hydrate loses all its water of hydration below 130° C. The glassy mass formed is very hygroscopic, this characteristic making it very difficult to obtain constant weight in drying dairy products and causing caking of the dried dairy products containing anhydrous lactose. At 150 to 165° C. lactose becomes yellow in color and at 175° C. it becomes brown, emits a characteristic odor and loses about 13 per cent of its original weight (151). This brown material contains anhydrous lactose, a substance insoluble in water, water-soluble lactocaramel and presumably other substances. The caramel may be isolated by first grinding the mass in warm alcohol and removing the lactose by filtration, and then evaporating the filtrate to a sirup, diluting with water and filtering again. This filtrate is evaporated to dryness and the residue dried at 100° C. The final product has a composition corresponding to $C_{12}H_{20}O_{10}$. A substance having the same empirical formula has been obtained by Pictet and Egan (153) by dehydrating lactose at 185° C. for 10 to 12 hours under vacuum. They believe it to be lactosan, i.e., galactosyl glucosan. It is insoluble in alcohol and has reducing properties. At 105° C. in the presence of zinc chloride it polymerizes to form a compound having no reducing properties. Hann and Hudson (146) by pyrolytic distillation under vacuum have obtained 6.5 grams each of a d-glucosan and d-galactosan from 100 grams of lactose.

OXIDATION PRODUCTS

The products obtained by the oxidation of lactose depend on the oxidation potential and concentration of the oxidant used, on the temperature and pH value of the reacting mixture and possibly on other factors.

Potassium permanganate oxidizes lactose in either acid or alkaline solution quantitatively to carbon dioxide and water (175, 176). Oxidation by nitric acid may yield mucic and saccharic acids quantitatively or, if the acid is sufficiently concentrated or hot, it may cause further oxidation to tartaric, racemic, oxalic and carbonic acids (165, 169). One product of the oxidation of lactose by chromic acid is furfural (160). Bromine oxidizes lactose in solution to lactobionic acid. In the presence of a buffer this is the only product, but without buffer, the hydrobromic acid formed causes hydrolysis, the final products being gluconic and galactonic acids (162, 166, 167). Beta lactose is much more rapidly oxidized by bromine than is alpha lactose (168). Iodine reacts with lactose under pressure at 100° C. to give formaldehyde, formic acid and a humic substance containing iodine (182). Neutral hydrated cupric oxide oxidizes lactose to a mixture of formic, lactic and glycollic acids (164).

Hydrogen peroxide has a negligible action on lactose unless an activating substance such as ferrous sulfate is present, in which case lactobionic acid is formed (159, 181). Ozone has no effect on lactose in acid solution; in alkaline solution its action is like that of air (163, 181).

The oxygen of air has no detectable action on lactose solutions unless they are alkaline, in which case it seems probable that salts of lactose are involved (177). In the presence of alkali and such catalysts as cerous hydroxide, ferrous sulfate, sodium sulfite, or sunlight plus zinc oxide as sensitizer, the sugar is oxidized to carbon dioxide and water (179). Without catalytic agents alkali induces auto-oxidation and degradation of lactose, as shown by changes in rotation (158), by the formation of acetic and formic acids (161), and of the saccharinic acids—tetrahydroxycaproic acids—studied extensively by Nef (178) and by Kiliani (170–174). Heating lactose with dilute sulfuric acid causes auto-oxidation to formic acid and beta-acetylpropionic acid (180).

HYDROGENATION PRODUCTS

The earliest attempt at hydrogenation of lactose was by Bouchardat (183) who used sodium amalgam and a lactose solution, obtaining a mixture of dulcitol, mannitol (?), sodium lactate, isopropanol, ethanol and hexanol. Neuberg and Marx (187) used calcium amalgam in an atmosphere of carbon dioxide, thus hindering secondary reactions, and obtained a crystalline product that was apparently the lactose alcohol, lactitol, 4-d-sorbitol-β-d-galacto-pyranoside, also called lactobiotol and lactositol.

Lactose in aqueous solution was treated by Ipatieff (185) with hydrogen under 74 atmospheres pressure at 130° C. in the presence of a catalyst of nickel and nickel oxide. The only product identified was dulcitol. Senderens (188) and Tanno (189) by substantially the same procedure, except that the catalyst was nickel only, obtained a mixture of lactitol, dulcitol and sor-

bitol. With a hydrogen pressure of only 30 atmospheres and a nickel catalyst, Karrer and Büchi (186) obtained amorphous lactitol and found its specific rotation $[\alpha]_D = +14.8°$ in water. Wolfrom *et al.* (190) treated a slightly acid lactose solution with hydrogen and reduced nickel at a temperature varying from 143 to 150° C. and a pressure from 102 to 138 atmospheres and obtained crystalline lactitol in 80 per cent yield. Their product had a melting point of 146° C. and a specific rotation $[\alpha]_D^{25°} = +14°$ in water. They prepared from it a crystalline tritrityl lactitol hexaacetate, interesting because of its high molecular weight of 1323. Hales (184) has obtained lactitol by electrolytic reduction of an acidified aqueous solution of lactose.

Zartman and Adkins (191) heated lactose, ethanol and hydrogen in the presence of a catalyst of chromium oxide and copper at a temperature of 250° C. and a pressure of 300 atmospheres. This procedure they designated hydrogenolysis, since the product, fractionated under reduced pressure, yielded methanol, more ethanol than used in the process, water, 1, 2-propanediol and three hydroxy compounds tentatively identified as 2-(4-hydroxytetrahydrofuryl)-methyl carbinol, a hexane triol and a hexane tetrol.

SUBSTITUTION PRODUCTS

Many more or less complex derivatives of lactose have been described in the chemical journals (14), but, since most of them have little practical interest, they will be mentioned only briefly here, the reader being referred to the cited papers for details of preparation or properties.

Lactose undergoes the characteristic sugar reaction with phenylhydrazine to give first the phenylhydrazone (204) and finally the osazone (203, 231). Several substituted phenylhydrazones of lactose have been prepared (202). Several lactose nitrates have been reported (213), the most definitely authenticated being the octonitrate (243), which has practical value as an explosive (199).

Acetylation of lactose produces heptaacetyl acetyllactoside (200, 217, 219, 238) which, treated with hydrogen bromide, gives heptaacetyl bromolactoside (200, 208). The other halogens may be introduced in a similar manner (195, 206, 216, 229). The reaction of heptaacetyl halogenlactosides with other substances has been used to synthesize other heptaacetyl lactosides (192, 197, 198, 207, 210, 211, 212, 215, 220, 233, 237). Deacetylation converts these compounds to simple lactosides (201, 207, 210, 212, 216, 221, 233). Reduction of heptaacetyl bromolactoside followed by deacetylation yields lactal (193, 209), a substance convertible to an iso and a pseudo modification and to a hydrolactal. Heptamethyl lactosides (26) and heptapropionyl propionyllactoside (222) have been prepared.

Lactose combines directly with amino compounds (223–228, 232, 235, 244). It forms a cyanhydrin which may be converted to lactose carboxylic acid (205, 214, 234). Some other compounds of lactose of possible interest

are the benzoates (230, 240), butyrate (194), butylmercaptan (242), phosphates (241), and its combination with sodium (218), cysteine (236) and calcium carbonate (239).

FERMENTATION PRODUCTS

General discussion. The products of fermentation of lactose are not peculiar to this sugar, since they are determined by the organism rather than by the substrate and all organisms that ferment lactose ferment other common sugars. On the other hand, many organisms that ferment other common sugars do not ferment lactose. Consequently the chief factors determining whether lactose shall be used as the carbohydrate for a commercial fermentation are the existence of an organism that will convert lactose efficiently into the desired product and the cheapness of whey relative to that of other available sources of fermentable carbohydrate, such as cane, beet or sorghum molasses or corn sugar. The presence of certain vitamins in whey gives it an advantage in some instances.

Of the many substances reported to have been isolated from the products of fermentation of whey, those produced in yield sufficient to warrant consideration for commercial exploitation are ethyl and butyl alcohols, butyric (246, 250, 251), citric (260), acetic (258), propionic (259), and lactic (254) acids, acetylmethylcarbinol (252), riboflavin and penicillin. Lactic acid, penicillin and riboflavin are being produced in this country by fermentation of lactose. The production of ethyl alcohol is possible in the future, but it seems unlikely that the other compounds listed will be made commercially by fermentation of whey.

Riboflavin. Clostridium acetobutylicum, an organism of the type used to produce butanol and acetone from corn mash, may, by proper culturing, be "trained" to produce riboflavin efficiently from sugar substrates (248, 255, 261). This procedure has been adapted to increasing the riboflavin content of whey to such an extent that the product will contain as high as 1000 micrograms of riboflavin per gram of solids. Details of the process are not yet published.

Ethyl alcohol. Although the production of ethyl alcohol from molasses is a firmly established industry and whey is, as a rule, a more costly source of fermentable carbohydrate than is molasses, the production of alcohol from whey in small inland communities with possible further conversion to a whey or spirit vinegar (258) is not economically out of the question. Recently, several articles have appeared (247, 249, 256, 257, 258) and patents have been issued (245, 253), on the production of ethyl alcohol from whey. The techniques described are similar to those for the fermentation of molasses, except that a lactose-fermenting yeast of high fermentative efficiency must be employed instead of the common *Saccharomyces cerevisiae.*

Lactic acid, manufacture. Many organisms are able to convert sugars to lactic acid, but for the production of lactic acid from the lactose of whey

a mixed culture of a lactobacillus and a mycoderm is used. This is commonly called "ga" and designated in the American Type Culture Collection as No. 9223. This culture converts lactose to lactic acid with an efficiency of over 95 per cent, the acid being the racemic mixture of the dextro and levo acids, and forms no objectionable byproducts. It grows readily at 43° C. and in a pH range of 5.0 to 5.8, conditions that effectively discourage the growth of organisms that might be present in whey and produce other products, such as, for example, butyric acid (268).

The process in commercial use is substantially that described below (262, 263, 264, 267).

The starter culture is built up by two successive inoculations into increasing quantities of sterilized skim milk and a third inoculation into 500 gallons of pasteurized whey. These cultures are incubated for 24 hours at 43° C. before being used as inoculum for the next larger volume of skim milk or whey.

The fermentation is conducted in a wooden tank of a total capacity of 6000 gallons. The tank should be provided with a perforated steam pipe for heating the whey, a portable agitator for intermittent use and an outlet of adjustable level for decanting.

After the tank is cleaned, chemically sterilized and rinsed free of sterilizer, 5000 gallons of whey are run in and heated to 43° C. The 500 gallons of starter are run in and the fermentation allowed to proceed for about 42 hours, heat being supplied, if necessary, in order to hold the temperature at 43° C. Every six hours, or at shorter intervals if convenient, a slurry of slaked lime is added to neutralize part of the acid and bring the reaction to the most favorable pH range. The lime used should be practically free of magnesia. It should be added gradually with the agitator running until the reaction is at about pH 6.0. At higher pH values there is risk of contamination, and below 5.0 the fermentation is considerably retarded. The pH value may be determined by means of bromocresol green paper or indicator solutions. The completion of the fermentation may be determined by a test for residual sugar with Fehling's solution or judged by the quantity of lime consumed.

The fermented whey is neutralized (pH 6.5–7.5) with lime slurry, heated nearly to the boiling point and held at that temperature for ten minutes or until coagulation of protein is complete. The coagulum is allowed to settle and the clear liquid decanted and run to a filter press, the sludge following. The hot filtrate is pumped to a wooden tank and a small percentage of decolorizing carbon added. The mixture is agitated and brought to a pH value of 10 by the addition of lime slurry, agitation being continued until a sample removed from the batch will sediment rapidly. It is then run to a filter press. The clear filtrate is neutralized by means of lactic acid and, either with or without another carbon treatment, depending on the quality

of product desired, concentrated in a vacuum pan to 15° Bé. The liquid is pumped to crystallizers lined with stainless steel and jacketed for circulating cold water. The mass is cooled to 10° to 15° C., and after about 12 hours standing crystallization is complete.

The crystalline mass is dropped to a centrifuge, the basket spun until no more liquor separates, the crystals washed lightly with water and spun until no more filtrate is obtained. The mother liquor and washings are evaporated to 13.5° Bé. and a second crop of crystals obtained. A third crop may be obtained before it is necessary to discard the liquor and washings.

The wet crude calcium lactate is dissolved in a glass-lined tank in the minimum quantity of water at 65° C., treated with carbon and filter aid, filtered, evaporated to 11.5° Bé., crystallized, centrifuged and washed. This treatment may be repeated, if necessary, in order to produce calcium lactate of U.S.P. grade. The mother liquors and washings are added to the crude lactate liquors.

Calcium lactate solutions of any degree of purity may be converted to lactic acid or lactates of other metals of corresponding grade. Sodium sulfate or carbonate may be used in making sodium lactate, the sulfates of iron and copper in making their lactates. A solution of one of these salts is mixed with a solution of calcium lactate in equivalent proportions, the insoluble calcium carbonate or sulfate removed by filtration and the filtrate concentrated by evaporation or evaporated to dryness.

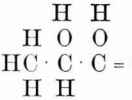
For conversion to lactic acid, sulfuric acid in very slight excess is added to a solution of calcium lactate of approximately 13.5° Bé. in a wooden or ceramic-lined tank. Decolorizing carbon and the calculated amount of potassium ferrocyanide sufficient to precipitate heavy metals present may be added, if acid of one of the better grades is desired. The insoluble calcium sulfate, carbon and ferrocyanides of the heavy metals are removed by filtration through a ceramic vacuum filter. The filtrate, which should contain about 25 per cent lactic acid, may be diluted to 22 per cent and marketed at this concentration or concentrated and subjected to further purification.

Concentration of lactic acid may be accomplished by evaporation in a stainless-steel vacuum pan or, up to over 50 per cent concentration, by a process known as "building up." This latter process consists of dissolving calcium lactate in lactic acid of 25 per cent concentration, made as described above, adding sufficient sulfuric acid to convert the dissolved calcium lactate to lactic acid, filtering, and then adding to the filtrate another increment of calcium lactate and repeating the cycle until the desired concentration of acid is obtained.

Lactic acid is marketed mostly as dark or light, 22 or 44 per cent acid, as colorless, edible 50 per cent acid, and as 85 per cent acid of U.S.P. grade.

Lactic acid, properties. The combination of properties that gives lactic acid a somewhat unique position from the standpoint of utility is shared only

by other alpha-hydroxy acids, of which glycollic acid is the only one produced in commercial quantities. The presence of alcohol and acid groups in the same molecule gives lactic acid,



The proximity of the alcoholic and acidic hydroxyls on two adjacent carbon atoms leads to the formation predominantly of linear polylactylic esters as water is progressively removed from lactic acid. Inner lactones are not formed under these conditions; the double ester, lactide, is formed in very small proportions and can be prepared only by a slow distillation process involving the dehydration of the dimeric acid. All solutions of lactic acid of concentrations greater than 20 per cent contain linear lactylic esters, which increase in both proportion and average chain length with increase in the gross concentration of the acid. As these linear polyesters increase in complexity, they become less susceptible to hydrolytic depolymerization, more viscous and more resinous in character (269, 270, 272, 273, 274, 276).

The heating of calcium lactate forms salts of dilactic acid, which is a result of loss of water from the alcohol groups of two lactyl units to form an ether cross-linkage (275).

Lactic acid, uses. The principal uses of lactic acid are in the leather industry, where it is used to neutralize the lime in limed hides (285), in foods, such as pickles, salad dressings, carbonated beverages and sherbets (289, 290, 302, 308, 318, 324) because of its clean acid taste and preservative action, and in alkyd resins (288, 295, 299, 300, 309, 321, 325) because of properties discussed above. Acid calcium lactate is used in Europe in baking powders (280, 287, 297, 298, 305, 306, 307, 315, 327). The ferrous and copper salts are used nutritionally. Sodium lactate solutions have been substituted for glycerol in textile printing and in paper-making (279) and various metal lactates have found use as mordants (296, 312, 326). Many esters of lactic acid have been made for use as solvents and as plasticizers (278, 282, 283, 284, 286, 293, 294, 300, 301, 303, 310, 311, 317, 319, 322, 323). Lactic esters are useful as an addition in molding cellulose ester compositions (277), and as starting materials for the production of the corresponding acrylates (281, 283, 291, 304, 313, 314, 320).

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OBSERVATIONS ON THE EFFECTS OF AN ANTERIOR PITUITARY PREPARATION ADMINISTERED TO LACTATING DAIRY COWS*

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In a previous publication Sykes, Meuleman and Huffman (4) reported that both the amount and percentage of fat in the milk of dairy cows were increased with injections of a relatively crude extract of the anterior pituitary gland. The injections were only given for a short period of time (5 days) and no detailed analysis of the milk was made. It was, therefore, thought desirable to give the injections for a longer time and to examine the milk for possible changes in constituents other than fat.

PROCEDURE

An alkaline extract of the anterior pituitary gland was prepared according to the method of Best and Campbell (1) and used in this study. All injections were given subcutaneously for periods ranging from seven to twenty-three days. Five hundred milligrams of the extract were given at each injection. Four Holstein cows in declining lactation were used in the initial studies, and later the extract was given to an ovariectomized lactating ewe and to a freemartin heifer which had been brought into lactation with hormone treatment.

Daily milk samples were obtained for analysis by mixing equal portions of morning and evening milk. The fat per cent and total solids were determined by the methods of Mojonnier and Troy (2). Chlorides were determined by the method of VanSlyke and Sendroy (5) and lactose by the official A.O.A.C. method (3).

RESULTS AND DISCUSSION

All four cows showed increases in the per cent fat of the milk. The initial increases occurred within three to five days after the first injection. *Cow 264* showed an initial increase during the first seven injection days from 4.5 to 5.8 per cent fat, a decline during the next three days to 4.8 per cent and then showed a gradual increase during the remaining thirteen days to attain a level of 10.1 per cent. Two days after injections were stopped this cow developed a severe anemia due to internal hemorrhage and died five days later. Milk production began to decline markedly two days before injections were stopped and the very high terminal fat per cent (10.1) was probably partly due to this fact.

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The milk fat of *Cow A 29* increased from 3.6 to 4.8 per cent on the third day of injection and maintained an average of 4.5 per cent for an additional nine days at which time the fat per cent rapidly fell to 2.0 per cent during the next four days, the udder swelled and ropy and curdy milk was produced. The injections were stopped at this time.

Cow D 9 increased from a pre-injection milk fat average of 3.9 per cent to an average of 4.3 per cent for a period of nineteen days of injections at which time the per cent fat decreased over a five-day period to 3.5 per cent. Injections were stopped at this point.

The milk fat of *Cow 290* increased from an average of 4.3 per cent to 5.5 per cent on the fifth day of injection. At this point the udder swelled, ropy milk was produced and lactation was terminated three days later at which time the fat per cent had decreased to 4.5 per cent.

It will be noted that the fat content of the milk of three of these cows (A 29, D 9, and 290) decreased after an initial increase in spite of continued injections. The fourth cow showed an abnormally high fat content during the last few injection days. Coincident with the drop in fat per cent, the udder of two cows (A 29, 290) became abnormal.

In general, as long as the fat per cent remained within reasonably normal limits and as long as the udders appeared normal, the changes in the other milk constituents tended to parallel the changes in fat with the exception of chlorides which showed either little change or a slight decrease. Moderate increases in solids-not-fat and lactose accompanied the increase in fat. However, when the udders of A 29 and 290 became abnormal and when the fat content of 264 had increased to about 6.0 per cent very marked changes occurred in the other milk constituents and marked irregularities in their concentration occurred from day to day in three of the four cows. The fat, solids-not-fat and lactose decreased markedly and then tended to return to normal levels when injections were stopped. All the constituents and particularly the lactose varied considerably from day to day, a condition which persisted for nearly a month after injections were terminated. The chloride concentration was generally inversely proportional to the lactose level. D 9 did not react in this manner and, in fact, did not respond as well in respect to increase in fat as did the other cows. This cow also differed from the other three in that she was not pregnant.

The level of production in all cows remained equal to or was slightly above the pre-injection level during the time the initial fat increases occurred, but declined rapidly when the udders became abnormal. The changes which occurred in the milk of A 29 are generally typical of the changes which occurred in the group as a whole. These are presented in figure 1.

In addition to the above changes it was found that all four cows developed cystic ovaries. Three cows developed multiple cysts of both ovaries and the fourth cow developed cysts on the right ovary. None of the three

pregnant cows terminated a normal pregnancy. Mummified fetuses were removed from two of the cows. The third cow (264) died, as mentioned previously, and it is not possible to state definitely whether mummification of the fetus had commenced although there were indications that such was the case.

These latter observations suggested that the increase in fat percentage and other changes in the milk of these cows was possibly due to the intense ovarian stimulus which the extract produced. Since the extract is known to possess gonadotropic hormone and since ovarian effects accompanied the changes in fat content of the milk, it seemed possible that this hormone might

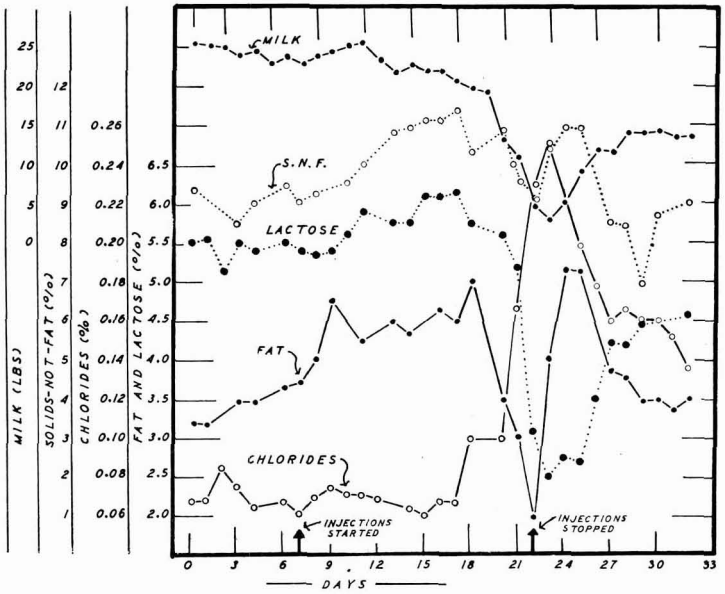

FIG. 1. Changes in milk constituents following injections of an anterior pituitary extract.

indirectly affect fat metabolism through ovarian stimulation. If such were the case it would not be necessary to postulate the existence of a separate fat metabolism hormone of the pituitary gland at least insofar as such a hormone affects the fat content of milk.

The extract was therefore administered to a lactating ewe (250 mg. daily) which had been ovariectomized two weeks previous to the first injection and to a freemartin heifer (500 mg. daily) which had been brought into lactation by prolonged treatment with estrogens and progesterone followed by prolactin. At autopsy the ovaries of this heifer were practically nonexistent and showed no signs of activity whatsoever.

The fat content of the milk of these two animals increased as a result of the injection of the extract in a manner similar to the cows. These results are shown in table 1. While the data are not extensive they suggest that the fat increase in the milk of cattle injected with this extract of the pituitary was not due to the ovarian stimulation. Unpublished results also indicate that the increase in liver fat which the extract produces in guinea pigs occurs just as readily in ovariectomized as in normal pigs. While it is possible that the gonadotropic hormones may have some direct effects on fat metabolism not mediated through the ovaries, there is at present no evidence

TABLE 1

The effect of an anterior pituitary extract on the per cent fat of the milk of an ovariectomized ewe and a freemartin heifer

Day	Fat content of milk		
	Ovariectomized ewe	Freemartin heifer	
	per cent	*per cent*	
1	9.5	3.7	No injections
2	8.6	3.9	"
3	10.0	3.4	"
4	8.0	3.0	"
5	8.7	.....	Injection
6	9.7	3.7	"
7	10.5	.....	"
8	10.7	3.7	"
9	10.5	3.4	"
10	15.0	3.6	"
11	11.7	3.6	No injections
12	15.3	4.4	"
13	19.5	4.3	"
14	15.5	3.6	"
15	12.5	4.0	"
16	12.5	.....	"
17	8.5	4.4	"
18	6.8	.....	"
19	.....	4.2	"

for this and the effects on fat metabolism produced by the extract used in these experiments would seem to be due to some other hormone, possibly a separate fat metabolism hormone.

SUMMARY AND CONCLUSIONS

Injection of an alkaline extract of the anterior pituitary gland into lactating cows produced an initial increase in the per cent fat of the milk and moderate increases in lactose and solids-not-fat. The chloride content was generally inversely proportional to the lactose level.

Continued injections produced even more marked changes. Generally, the fat, lactose and solids-not-fat declined to very low levels and the chloride increased. Marked irregularities occurred from day to day. These latter effects have not been observed to occur earlier than the fifth day of injection

and in one animal injections given for twenty-four days failed to produce them. Swelling of the udder and ropy milk accompanied these changes.

The extract likewise produced cystic ovaries in all the cows to which it was given and mummified fetuses were removed from two of the three pregnant animals used following the injection period.

Preliminary work indicates that the changes in fat percentage of the milk were not due to the ovarian stimulation which was produced by the extract and it would appear that these changes were due to some hormone other than the gonadotropins contained in the extract.

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THE INFLUENCE OF A SYNTHETIC THYROPROTEIN WHEN FED TO DAIRY COWS OVER A THREE-WEEK PERIOD*

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INTRODUCTION

Graham (5) was the first to observe that the thyroid gland has a profound influence on the level of milk and milk-fat secretion of cows. He (6) later identified the active principle of the thyroid gland as thyroxine. These observations have since been confirmed by a number of investigators (7, 8, 1, 2, 3, 14, 9, 4).

Although the thyroxine studies added much to our knowledge of the factors controlling the level of milk and milk-fat secretion, no practical application of the results was possible because of the excessive cost of thyroxine. Recently Reineke and Turner (13) have made it more practicable by the formation in vitro of highly active thyroproteins. Reports on the effect of feeding such thyroproteins to dairy cows have also appeared (12, 10, 13, 11).

This work was begun to determine whether or not it is possible to increase the fat content of milk, without greatly augmenting milk production, by feeding a moderate daily dose of thyroprotein.

EXPERIMENTAL PROCEDURE

Five dairy cows in varying stages of declining lactation and gestation were selected for the experiment (table 1). Available production records at the beginning of the experiment indicated that the cows were secreting milk with a fat content below that of their respective breed averages.

TABLE 1

Description of cows fed a synthetic thyroprotein for a three-week period

Cow No.	Breed	Age Yr.—Mo.	No. of lactation	Month of lactation	Month of gestation
H–41	Holstein-Friesian	3	1st	10th	7th
H–37	"	4—11	2nd	8th	3rd
627	Brown Swiss	3— 5	1st	5th	1st
492	Ayrshire	3— 5	1st	6th	5th
383	Jersey	3— 1	1st	4th	0

The experiment consisted of a two-week pre-experimental period, a three-week experimental period, and a two-week post-experimental period. Ten

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grams of a thyroprotein (Protamone[1]) were fed daily in the grain ration during the three-week experimental period. During the entire course of the experiment daily milk weights (twice daily milkings) were recorded and milk samples were taken on two consecutive days each week. Individual milk samples were tested for their butterfat content (Babcock method) and solids-not-fat content (by means of a lactometer). Body weights and heart rates (by means of a stethoscope) were determined on two consecutive days each week.

EXPERIMENTAL RESULTS

Fat test. The fat test, on the average, increased from 3.62 to 4.11 during the feeding period and then declined to 3.76 in the second week of the post-experimental period. Increases in fat test during the course of the entire experiment varied from 0.34 to 1.51, the latter increase being observed in the cow most advanced in lactation. It is interesting to note that the withdrawal of Protamone from the ration of a cow (H–41) advanced in lactation, as well as cows not so far advanced in lactation, resulted in a decrease in fat test. The decrease in fat test in the second week of the post-experimental period is indeed remarkable since it occurs at a time when milk production is decreasing. The results are summarized in table 2.

TABLE 2

Influence of feeding 10 grams of Protamone daily on the fat content of milk

Period	No. of days	Cow number					Weighted ave.
		H–41	H–37	627	492	383	
		% fat	*% fat*	*% fat*	*% fat*	*% fat*	*% fat*
Pre-experimental	14	3.31	3.03	3.79	3.29	4.80	3.62
Experimental	7	3.24	3.08	3.96	3.15	4.58	3.61
	7	3.63	3.30	4.15	3.68	4.88	3.90
	7	3.58	3.37	4.25	4.25	5.23	4.11
Post-experimental	7	4.82	3.27	4.17	3.87	5.25	4.13
	7	3.67	3.10	3.90	3.57	5.02	3.76
Maximum increase during experiment	.....	1.51	0.34	0.46	0.96	0.45	0.51

Milk production. Protamone feeding stimulated varying increases in milk production. Based on weekly averages, the maximum increase in milk production was 1.7 lbs. per day. Increases in individual cows (weekly averages) varied from 0.5 to 4.7 lbs. Two cows (H–41 and 383) attained their highest average daily production during the first week of the feeding period while two other cows (H–37 and 627) did not reach their highest average

[1] The Protamone was generously supplied by the Cerophyl Laboratories, Kansas City, Missouri, through the courtesy of Dr. W. R. Graham, Jr.

daily production until the third week of the feeding period. The cow (H–37) showing the greatest increase in milk production showed the least increase in the fat content of her milk and conversely the cow (H–41) giving the smallest increase in milk production showed the greatest increase in the fat content of her milk, the latter being most advanced in her lactation period.

On the removal of Protamone from the ration there was a slight decrease in milk production. Nevertheless the level of milk production during the post-experimental period appeared to be about the same as it would have been had Protamone not been fed (table 3).

TABLE 3

Influence of feeding 10 grams of Protamone daily on milk production

Period	No. of days	Cow number					Ave.
		H–41	H–37	627	492	383	
		lbs. milk per day	*lbs. milk per day*	*lbs. milk per day*	*lbs. milk per day*	*lbs. milk per day*	*lbs. milk per day*
Pre-experimental	14	15.2	29.3	26.1	23.5	22.3	23.3
Experimental	7	15.7	31.7	25.8	25.6	25.6	24.9
	7	15.3	33.1	27.7	25.8	23.2	25.0
	7	14.6	34.0	28.4	24.9	22.9	25.0
Post-experimental	7	13.0	31.2	25.2	23.0	20.3	22.5
	7	10.3	29.0	23.3	21.1	16.8	20.1
Maximum increase during experiment (weekly average)	.....	0.5	4.7	2.3	2.3	3.2	1.7

Solids-not-fat. The solids-not-fat content of the milk did not appear to be affected by Protamone feeding. The five cows showed an increase in the solids-not-fat content of their milk during the experimental period but it is doubtful if this increase can be attributed to the feeding of Protamone since only 2 of the 5 cows showed a decrease in the solids-not-fat content of their milk in the post-experimental period. The average figures suggest that the observed increase in solids-not-fat content can be attributed to the advance in the stage of lactation (table 4).

Body weight. There was a slight loss in body weight in four of the five cows. The cow that did not lose in body weight was the one (H–41) most advanced in lactation and pregnancy. The greatest loss in body weight occurred in the lightest cow of the group and this would be expected since Protamone was not fed on the basis of body weight. In no instance, however, can the loss in body weight be considered severe.

There was a marked increase in body weight in the first week of the post-

experimental period. This gain in body weight is associated with a decrease in milk production and in heart rate. In the second week of the post-experimental period four of the five cows showed a slight loss in body weight and this loss in body weight was associated with an increase in heart rate. The cow (383) that continued to show a gain in body weight during the second

TABLE 4

Influence of feeding 10 grams of Protamone daily on the solids-not-fat content of milk

Period	No. of days	Cow number					Weighted Ave.
		H–41	H–37	627	492	383	
		% S–N–F	% S–N–F	% S–N–F	% S–N–F	% S–N–F	% S–N–F
Pre-experimental	14	8.32	8.13	9.13	8.87	9.24	8.74
Experimental	7	8.49	8.19	9.14	8.90	9.30	8.80
	7	8.83	8.29	9.14	9.16	9.40	8.96
	7	8.73	8.36	9.38	8.94	9.15	8.91
Post-experimental	7	8.97	8.27	9.28	9.14	9.55	9.04
	7	8.95	8.40	9.35	9.11	9.59	9.08

week of the post-experimental period was the one that had lost the most in body weight during the experimental period and the one that showed the greatest decrease in milk production during the post-experimental period. The body weight averages are presented in table 5.

TABLE 5

Influence of feeding 10 grams of Protamone daily on body weight

Period	No. of days	Cow number					Ave.
		H–41	H–37	627	492	383	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Pre-experimental	14	1170	1219	1210	1081	791	1094
Experimental	7	1187	1204	1219	1100	777	1098
	7	1192	1242	1208	1086	781	1102
	7	1178	1212	1189	1077	755	1082
Post-experimental	7	1245	1310	1272	1138	781	1149
	7	1241	1279	1240	1119	816	1139
Maximum decrease in body weight	.....	.....	15	21	4	36	12

Heart rate. At the end of the second week of the experimental period heart rate had increased from 74 beats per minute to 83 beats per minute. All of the cows showed increases in heart rate, with the increases varying from 8 to 14 beats per minute. The cow (492) with the lowest initial heart

rate had the greatest increase in heart rate (14 beats per minute) while the cow with the highest initial heart rate (H–37) showed the least increase in heart rate (8 beats per minute).

In the third week of the feeding period heart rate was similar to that of the pre-experimental period. This decrease in heart rate cannot be accounted for, but it was not caused by temperature changes. That the Protamone was stimulating heart rate during the third week of the experimental period is attested by the fact that there was a further decrease in heart rate during the post-experimental period (table 6).

TABLE 6

Influence of feeding 10 grams of Protamone daily on heart rate

Period	No. of days	Cow number					Ave.
		H–41	H–37	627	492	383	
		Rate per minute	*Rate per minute*	*Rate per minute*	*Rate per minute*	*Rate per minute*	*Rate per minute*
Pre-experimental	14	71	82	76	68	74	74
Experimental	7	72	84	88	80	85	82
	7	83	90	77	82	84	83
	7	73	75	71	71	76	73
Post-experimental	7	70	64	58	64	55	62
	7	72	70	60	68	76	69
Maximum increase in heart rate	.....	12	8	12	14	11	9

DISCUSSION

It appears that the feeding of Protamone in a moderate daily dose will definitely increase the butterfat content of milk. This increase in butterfat content is not accompanied by either any great increase in milk production or a great loss in body weight. There are no objections to increases in milk production; in fact, they are most desirable, provided they can be obtained without encountering severe losses in body weight. Certain cows are good milk producers but low testers. Obviously such cows should not be fed large amounts of Protamone, for that would undoubtedly result in severe losses in body weight. If, however, moderate doses of Protamone will increase fat test, and this experiment indicates that they will, then it is certainly advantageous to feed Protamone. On the other hand, many cows increase in body weight at the expense of milk production, and such cows should receive larger doses of Protamone.

SUMMARY

The feeding of 10 grams of Protamone daily for 3 weeks to a group of 5 dairy cows increased the butterfat content of the milk from 3.62 per cent to 4.11 per cent. The average milk production was increased from 23.3 lbs. to

25.0 lbs. per day. Losses in body weight were slight and heart rate increases were moderate. Solids-not-fat did not appear to be affected.

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A CONTROLLED EXPERIMENT IN FEEDING WHEAT GERM OIL AS A SUPPLEMENT TO THE NORMAL RATION OF BULLS USED FOR ARTIFICIAL INSEMINATION[1]

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Though the importance of adequate vitamin E in the rations of rats for normal reproduction is well known (7), no clear-cut evidence has been obtained that the reproductive performance of the larger farm animals is affected by insufficient vitamin E. In 1931 Vogt-Moller and Bay (18) reported that injections of wheat germ oil at the time of heat, or just before, were effective in curing sterility in some cows, which had failed to settle on repeated earlier services, but which otherwise appeared normal. Later (2) these same investigators reported continued success with their uncontrolled treatments. In controlled studies with a limited number of cows Asdell *et al.* (1) were unable to show that injections of wheat germ oil gave results different than those obtained with untreated controls. Gwatkin and MacLeod (4) showed that wheat germ oil therapy was ineffective in altering the course of *Brucella abortus* infection in a group of 12 cows compared with a similar group of controls.

Thomas and collaborators (14, 15, 16, 17) in extensive studies with goats and sheep have shown that in these species, at least, the vitamin E requirements are either very low or that a dietary supply of this vitamin is not needed. In these studies the vitamin E in the basal diet was destroyed by treatment with an ether solution of ferric chloride and aging. Normal reproduction of the goats and sheep proceeded on this ration which was so low in vitamin E that rats were unable to reproduce on it.

Titus and Burrows (13) have determined the effect on semen production of 0.5 per cent of wheat germ oil in experimental rations of cockerels. They found that wheat germ oil decreased semen production. No similar study with the larger farm animals has come to the attention of the writer.

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[1] The investigation was conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc., Syracuse, N. Y., who placed their bulls and other facilities at the disposal of the author, and in cooperation with the Viobin Corporation, Monticello, Ill., who supplied the wheat germ oil used. The cooperation of these parties is sincerely appreciated.

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Hathaway, Davis and Graves (5), Hathaway and Davis (6), and Palmer, Nelson and Gullickson (8) have determined in bio-assays with rats the vitamin E content of a number of the feeds commonly fed to dairy cattle. Their results indicate that most rations for dairy animals, composed of natural, common feeds and satisfactory in other respects, would contain liberal amounts of vitamin E for satisfactory reproduction in rats. This fact and the results of the other investigations cited raise the question of the value of supplementing a practical ration composed of natural feeds with wheat germ oil as a source of vitamin E to prevent sterility in dairy animals. In spite of these facts, the use of wheat germ oil as a supplement to the normal rations of breeding farm livestock, especially males, is quite widespread to judge from verbal reports received from livestock men.

In view of these facts it was considered desirable to determine the value of supplementing a normal ration composed of usual, common feeds for dairy bulls with wheat germ oil over a sufficiently long period of time to determine whether or not the added wheat germ oil had an effect on semen production and upon the fertility of that semen when used for artificial insemination.

EXPERIMENTAL

Twenty bulls were selected for the investigation from the 28 bulls then owned by the New York Artificial Breeders' Cooperative and housed in one barn near Syracuse, New York. These bulls were of the Holstein-Friesian and Guernsey breeds and were used only in artificial breeding. The 20 bulls were divided into two groups as nearly equal in all respects as could be determined. The basis of classifying the bulls into groups is given in table 1. This table gives the mean for each bull, the mean of the group and the standard deviation of the group means for each criterion used in the grouping. The relative fertility of the bulls was established before the experiment started by the proportion of the cows to which each bull was artificially bred during a three months' period, which did not return to service for at least one month after being bred. The data on average semen volume and average motility of the spermatozoa for each bull was obtained during the same period of time. On the other hand, the spermatozoa counts (table 1) are for but one collection taken during the week before the experiment started, and are less reliable than the other measures. The weight of each bull was estimated from heart girth measurements.

As can be seen from the table the two groups were very similar in the criteria used for classification. After the individual groups were selected a flip of a coin by a disinterested party determined that Group I would receive the wheat germ oil. This group of bulls was called the wheat germ oil-fed group, while Group II was known as the control group.

All bulls were fed the same concentrates and hay twice daily. Hay was the only roughage fed. It was composed of about 50 per cent alfalfa, 40 per

TABLE 1

Criteria for grouping bulls

Bull number	Breed*	Age	Estimated weight	Average volume semen	Spermatozoa count per mm.^{3}	Average motility	Services	Non-returns to service
Wheat germ oil-fed group (I)								
		mo.	*lbs.*	*ml.*	*thousands*	%		%
1E	H	40	1700	5.8	772	72	150	58.0
2E	H	109	2125	7.6	897	73	269	63.2
3E	H	123	2025	6.7	623	70	119	64.7
4E	H	20	1197	3.5	1,178	74	113	77.9
5E	H	90	1791	5.1	963	66	78	76.9
6E	H	51	2075	5.4	1,783	77	100	62.0
7E	H	13	853	2.0	1,377	60	28	57.1
8E	G	87	1745	7.1	718	70	144	64.6
9E	G	86	1791	8.1	1,412	78	149	65.8
10E	G	97	1538	9.0	1,945	80	98	70.4
Mean	.....	71.6 ± 38.0	1684 ± 400	6.03 ± 2.14	1,166.8 ± 454.2	72.0 ± 5.94	124.8 ± 62.8	66.1 ± 7.1
Control group (II)								
1C	H	37	1607	5.1	1,387	73	105	58.1
2C	H	98	2205	9.0	700	74	205	64.4
3C	H	121	1837	5.1	1,443	76	155	65.8
4C	H	25	1377	2.5	987	68	55	61.5
5C	H	78	2025	4.9	1,507	72	107	76.6
6C	H	66	2125	6.3	1,777	70	18	61.1
7C	G	17	908	2.9	1,123	66	107	66.4
8C	G	78	1607	7.2	703	81	112	55.4
9C	G	52	1630	7.7	803	68	131	71.8
10C	G	96	1607	7.5	1,129	76	68	60.3
Mean	.....	66.8 ± 33.9	1693 ± 383	5.82 ± 2.11	1,155.9 ± 367.1	72.4 ± 4.57	106.3 ± 53.4	64.2 ± 6.4

* H = Holstein-Friesian.
G = Guernsey.

cent or less of timothy, and about 10 per cent other grasses and clover. The hay varied somewhat throughout the experiment, but was largely of No. 2 grade. When the experiment was first started, April 15, 1941, hay grown the previous season was fed. After July 15, hay produced during that season was fed, and was continued to the end of the experiment, April 14, 1942.

The concentrate mixture was the one the Cooperative had been feeding for some time. It was made up of normal feeds ordinarily available to any dairyman and contained the following proportions of ingredients:

46.5	lbs. ground oats
18.5	lbs. yellow cornmeal
19.0	lbs. wheat bran
4.5	lbs. linseed meal
4.5	lbs. soybean oil meal
4.5	lbs. steamed bone meal
2.5	lbs. salt
100.0	

The mixture contained approximately 14 per cent total protein. The feed allowance of both concentrates and hay was weighed one day each week throughout the experiment. On this basis, the bulls in Group I received an average estimated daily allowance of 20.6 pounds of hay and 7.7 pounds of the concentrate mixture. The control group (II) received an estimated average of 20.8 pounds of the hay and 8.0 pounds of the concentrate mixture daily.

Three samples each of the hay and concentrate mixture were assayed with rats at different times during the experiment for vitamin E potency by Dr. Karl E. Mason. Female rats, critically depleted of vitamin E at the time of weaning, were subsequently reared upon an E-deficient diet modified by substituting a variable proportion of the test samples for a corresponding portion of the cornstarch component. The success or failure of their first pregnancy was used as a basis for determining the adequacy or inadequacy of the modified diet. With the first and third hay sample complete fertility response resulted when the hay made up but 20 per cent of the modified diet. For the second hay sample, 25 per cent was needed, and for each of the three samples of the concentrate mixture 30 per cent of the modified diet was necessary for complete protection.

One ounce of solvent process wheat germ oil[3] daily was fed to each bull in Group I. The oil was poured on the concentrate mixture at each night feeding. The oil was weighed out to each bull from one quart containers which were kept in the ice-box until completely used. A fresh supply of the oil was received at approximately one-month intervals.

[3] The Viobin Corporation report that the oil contained approximately 2 Evans rat units of vitamin E per gram.

The first nine bulls of both groups were started on the experiment on April 15. On June 1, one bull was added to each of the experimental groups. These are listed as bulls 10E and 10C in table 1. The experiment was continued through April 14th of the following year. Thus, nine bulls of each group were on the experiment from the beginning and, if they continued to produce semen of satisfactory quality, were expected to remain on the rations for one year. For the remaining bull in each group the planned duration of the experiment was for ten and one-half months.

Semen was collected from each bull in the experiment approximately once each week. Ordinarily the bull was sampled at each of these collection periods as many times as was necessary to obtain the required quantities of semen of high enough quality to give satisfactory assurance that the expected number of cows in heat could be impregnated by it. Often two ejaculations at a collection period were taken and sometimes three or more were collected from a single bull in one day. The collection of this many ejaculates from a bull in one day was due usually to the fact that the first collection of semen was so low in spermatozoa count or so low in spermatozoa motility that it was discarded. Each semen sample which, by the criteria used, gave indications of being satisfactory for artificial insemination, was diluted with the yolk-citrate diluent (10) and shipped to the various sections of New York State where it was used for inseminating cows. The dilution rates for the semen used in this experiment were similar for each group of bulls and varied from 1 part of semen to 2 parts of the diluter up to 1 part of semen to 16 parts of the diluter. The semen was diluted at rates largely dependent upon the number and activity of the spermatozoa in each sample. Salisbury *et al.* (11) have shown that similar rates of conception may be expected for semen diluted on this basis and within the range of dilution used in this investigation. One ml. of diluted semen was used for each insemination. The spermatozoa counts were made with the hemocytometer. The methods used to determine motility and in handling the semen before insemination were described earlier (19).

RESULTS

The results of the investigation are presented in two parts; the first deals with the breeding behavior of the bulls and the characteristics of the semen produced by them; the second deals with the fertility of the two groups of bulls when the semen was used for artificial insemination.

Breeding behavior of the bulls and semen characteristics. Table 2 presents the mean data relative to the semen characteristics measured of each group of bulls. These measurements were made throughout the entire experimental period. The time required for service was determined only during the first six months of the experiment and the mean time, also, is shown in table 2. This, so-called, "service time" is the number of seconds from the time the bull approached a fixed point about 4 feet behind the

"teaser" cow to the time of ejaculation. When more than one ejaculate was taken at the time of collection, the bull was removed some ten feet from the cow until preparations were completed for the second collection. This time interval varied from about 3 to 5 minutes.

In many cases, the semen by gross observation or as a result of the microscopic examination or spermatozoa count, was considered unfit for use in artificial insemination. Such samples were discarded and are so listed in table 2. In other cases it was found impossible for one reason or another

TABLE 2

Characteristics of semen produced by experimental bulls and their breeding behavior

	Ejaculate number	Wheat germ oil-fed group (I)		Control group (II)	
Total number of ejaculates collected	1	335		335	
	2	219		281	
	3	38		62	
	4	2		4	
	All	594		682	
Average volume of ejaculates, cc.	1	5.9	(312)	5.3	(301)
	2	6.4	(203)	6.6	(253)
	3	5.2	(34)	5.5	(55)
	All	6.0	(570)	5.7	(654)
Average motility of spermatozoa in ejaculates, per cent	1	71.0	(282)	69.8	(261)
	2	72.7	(184)	76.6	(231)
	3	72.2	(27)	74.9	(50)
	All	71.4	(533)	73.0	(606)
Average number of spermatozoa in ejaculate, 1,000's per mm.^{3}	1	1,323	(179)	1,259	(160)
	2	1,113	(115)	1,281	(170)
	3	1,099	(15)	1,008	(28)
	All	1,207	(409)	1,227	(480)
Average service time, seconds	1	193	(159)	108	(147)
	2	166	(98)	124	(120)
	3	105	(17)	78	(27)
	All	174	(302)	104	(353)
Total volume semen discarded, cc.	.....	652	(120)	620	(123)
Ejaculates discarded, volume unknown	.....		(15)		(25)

to obtain all of the information desired on each semen ejaculation. Occasionally, two successive ejaculates were taken in the same artificial vagina, without using two different collection tubes. The average data for such combined ejaculates are included in table 2 under the totals and explain the discrepancy found in the table for the number of samples which were used to obtain the average for all ejaculates and the sums of the number of ejaculates used for the average of the first, second and third ejaculates. Fourth ejaculates were so few in number that they are included in the average for all ejaculates only. In this table the data are an average of those samples for which observations were made and the number of ejaculates on which each average is based is included in parenthesis after the data. There was

no apparent difference between the experimental groups in this regard. From this fact it is believed that the semen produced by each group of bulls has been well sampled and that conclusions may be drawn from this table concerning the effects of the experimental treatments.

More individual ejaculates of semen were collected from the control bulls than was the case with the wheat germ oil-fed bulls. This was due primarily to the fact that the average volume of the first ejaculates, of which an identical number was collected from each group of bulls, was enough smaller for the control bulls so that, in order to have sufficient semen for breeding, it was more often necessary to collect a second ejaculate. This result was not surprising for the bulls in Group II produced a smaller quantity of semen in the first ejaculates during the three months preliminary period before the experiment started. In fact the mean volume of all ejaculates for the experimental period varied little from the mean volume of all ejaculates obtained during the preliminary period. In addition, there was a greater demand for the semen of these bulls as indicated by the fact that they were bred to 884 more cows during the experiment than were the bulls of Group I.

To test this apparent difference in semen volume statistically and to determine whether or not there were other differences between the experimental groups, the mean data for all ejaculates of each bull were arranged by months and an analysis of variance made. In each case the accepted method of analysis when unequal numbers are found in the groups was used (12). For semen volume, per cent of motile spermatozoa, spermatozoa count and number of discarded ejaculates there were no statistically significant differences between the wheat germ oil-fed and the control groups.

With respect to the time required for service, as a measure of the sex drive of the bulls, there was a highly significant difference in favor of the control bulls. The difference in actual time, however, was only slightly more than one minute and was not of great practical importance. Two bulls on each ration, Nos. 6E, 8E, 8C and 9C, were discarded from the experiment for failure to satisfactorily settle the cows to which they were artificially bred or for refusal to use the artificial vagina. One other bull on each of the rations was not used for various lengths of time during the experiment because of injuries which had nothing to do with the rations fed. Table 3 gives the pertinent data on the bulls which were removed from the experiment. The bull 10E which was not used from July 10 to November 1, because of lameness, was fed the experimental ration throughout this period. The other bull which was injured, 4C, did not recover from the badly bruised knee and was sold.

From the fertility data obtained before the start of the investigation, bulls 6E and 8E, though slightly lower in per cent of non-returns to service than the mean of Group I, were not expected to drop in fertility. After the

first few months on the experiment it was apparent that they were decreasing in fertility. For best field results in artificial insemination it would have been desirable to use them no further for breeding. However, it was desired to continue the wheat germ oil feeding over a sufficiently long period of time to determine whether they could be improved in fertility. When no improvement resulted, after 8 months in the case of one bull and 10 months with the other, they were removed from the experiment. They were both used for breeding during this period.

Bull 8C was the lowest in fertility of the control bulls during the preliminary period, though not so low that he was not expected to continue in service. This bull was used regularly for three and one-half months and so many of the cows to which he was artificially bred failed to settle that the

TABLE 3

Bulls removed from the experiment and reasons for removal

Bull number	Dates used	Reasons for removal	Spermatozoa count per mm.³	Motility	Fertility to date†		
					S	C	%
			Wheat germ oil-fed bulls				
			thousands	%			
6E	4/15/41 to 2/21/42	Low fertility	1,332 (35)*	74 (46)	364	143	39.3
8E	4/15/41 to 12/11/41	Low fertility	928 (27)	71 (34)	219	73	33.3
10E	6/ 1/41 to 7/10/41 and 11/ 1/41 to 4/14/42	Lameness—rested for about 3½ months					
			Control bulls				
4C	4/15/41 to 7/14/41	Bruised knee—sold for beef					
8C	4/15/41 to 4/ 4/42	Low fertility	752 (14)	57 (17)	61	24	39.3
9C	4/15/41 to 10/29/41	Refusal to use artificial vagina	1,970 (10)	67 (12)	125	65	52.0

* ( ) = ejaculates in average.
† S = services. C = conceptions.

semen was not used for insemination after July 30. Semen samples were collected on November 25, 1941, March 26 and April 4, 1942, but all were of such poor quality that they were not used for breeding cows. Bull 9C settled as many cows as the average bull in either group during the 6 months he remained in the experiment. However, he became reluctant to use the artificial vagina and finally refused to mount the teaser cow. He was kept on hand until February, 1942, but could not be induced to attempt to mount. It is believed that an injury was responsible for this condition.

Effect of season on semen characteristics. In table 4 are shown the average volume, spermatozoa count and per cent of motile spermatozoa in the

semen collected from each group of bulls arranged by months. These data show a tendency for the per cent of motile spermatozoa to be somewhat lower in the early spring months, and the concentration of spermatozoa to be lowest in August. No significant difference between months was found for service time, and for volume of semen, but for spermatozoa count and per cent of motile spermatozoa the differences between months were highly significant (< 1.0 per cent level of probability). For the number of ejaculates discarded the difference between months was just significant at the 5 per cent level of probability. These data indicate that the seasonal fluctuations in semen quality which occurred in central New York with the 20 bulls were not as great as those reported for 4 bulls in Indiana by Erb, Andrews, and Hilton (3), or for 6 bulls in Maryland by Phillips *et al.* (9).

Fertility of the bulls. The method used to determine the fertility of the bulls is presented in detail elsewhere (11). In brief, it consisted of deter-

TABLE 4

Effect of season on semen characteristics. Average of all ejaculates for which information was available

	Wheat germ oil-fed group			Control group		
	Volume	Concentration per mm.^{3}	Motility	Volume	Concentration per mm.^{3}	Motility
	cc.	*thousands*	%	*cc.*	*thousands*	%
April 15–30	6.3 (25)	.....	63.5 (23)	5.1 (31)	.....	59.3 (30)
May	5.8 (53)	1120 (48)	64.9 (51)	5.3 (58)	1094 (58)	65.4 (57)
June	6.2 (57)	1348 (49)	72.5 (54)	5.2 (62)	1436 (54)	73.0 (61)
July	6.2 (50)	1133 (50)	73.6 (54)	5.3 (69)	1128 (62)	77.0 (69)
August	5.6 (58)	977 (53)	76.9 (58)	5.6 (56)	1055 (46)	75.1 (54)
September	6.0 (48)	1078 (35)	76.0 (45)	5.9 (56)	1198 (51)	79.8 (55)
October	5.5 (28)	1338 (24)	73.1 (29)	5.6 (48)	1511 (36)	78.6 (46)
November	6.3 (52)	1247 (28)	71.2 (42)	6.3 (39)	1211 (22)	71.7 (33)
December	5.9 (50)	1241 (29)	69.8 (48)	5.5 (57)	1252 (33)	71.8 (53)
January	5.4 (48)	1287 (25)	76.0 (43)	6.4 (46)	1216 (30)	76.1 (46)
February	6.1 (41)	1474 (25)	67.6 (35)	5.8 (54)	1417 (23)	69.2 (51)
March	5.9 (44)	1312 (28)	66.9 (36)	6.0 (59)	1185 (49)	72.8 (43)
April 1–14	6.4 (16)	1260 (15)	68.7 (15)	6.3 (19)	1182 (16)	70.0 (8)

mining the proportion of the cows to which each bull was bred which did not return to service within a period of at least five months after service. Such cows were considered to be pregnant. This method varied from that employed to determine the relative fertility of the bulls during the preliminary period for, in this case, information regarding fertility was desired immediately prior to the start of the experiment and it was impossible to wait until the five months' period had elapsed.

The summarized data covering the entire experimental period is presented in table 5. In the original data the number of services and conceptions were summarized for each bull for each month. The percentage of the total services resulting in conception were then calculated and an unweighted

analysis of variance of the percentage figures, using the accepted methods for analysis when unequal numbers are found in the groups (12), was made. In a previous publication (11) it was felt necessary to use a weighted analysis because the per cent of services resulting in conception from individual ejaculates was under consideration. In this case the data for each month are a summary of several ejaculates and the extremely wide range in services per item observed in the basic data of the earlier study was not found.

The results of the statistical analysis of the fertility data show a significant (< 5.0 per cent level of probability), though small, difference in fertility in favor of the control bulls over the wheat germ oil-fed group. These results are not interpreted as indicating a depressing effect of wheat germ oil on the fertility of the bulls to which it was fed. However, no benefit to

TABLE 5

Fertility of the experimental bulls

Wheat germ oil-fed bulls				Control bulls			
Bull No.	No. Services	No. conceptions	% conceptions	Bull No.	No. services	No. conceptions	% conceptions
1E	379	170	44.9	1C	303	126	41.6
2E	611	320	52.4	2C	1024	601	58.7
3E	345	133	38.6	3C	641	294	45.9
4E	450	274	60.9	4C	86	58	67.4
5E	298	177	59.4	5C	889	412	46.3
6E*	364	143	39.3	6C	576	309	53.6
7E	121	54	44.6	7C	382	220	57.6
8E*	219	73	33.3	8C*	61	24	39.3
9E	460	244	53.0	9C*	125	65	52.0
10E	434	235	54.1	10C	478	273	57.1
Total	3681	1823	49.5	Total	4565	2382	52.2

* Dropped from experiment after varying periods of time for low fertility or refusal to use the artificial vagina.

fertility resulted from supplementing the ration composed of common, natural feeds with the wheat germ oil. The addition of wheat germ oil to the ration was ineffective in preventing a decrease in fertility in two bulls which finally had to be withdrawn from use in artificial insemination.

In spite of the fact that an effect of season on the quality of semen produced by the bulls was shown, the fertility of the same semen did not show correlated trends. The statistical analysis showed no significant difference in fertility between months.

SUMMARY

Two comparable groups of 10 bulls each were selected. Both were fed a practical ration made up of common, natural feeds which supplied plenty of vitamin E for normal reproduction of rats. To determine whether or not additional vitamin E in the form of solvent process wheat germ oil would benefit the reproductive performance of bulls used extensively for artificial

insemination, each of the bulls in one group received one ounce daily of the wheat germ oil during an experimental period of one year. Over 1,250 semen samples were collected from the 20 bulls, and over 8,200 cows were artificially inseminated during the experiment.

A comparison of the results from the two groups warrants the following statements:

The feeding of one ounce daily of solvent process wheat germ oil in addition to the normal ration did not:

1. Increase the volume of semen produced by the bulls;
2. Increase the spermatozoa concentration in the semen;
3. Improve the motility of the spermatozoa;
4. Shorten the time required for service;
5. Decrease the number of semen ejaculates which were discarded as being of too poor quality for use in artificial insemination;
6. Improve the fertility of the bulls to which it was fed;
7. Nor prevent two bulls from decreasing in fertility to such low levels as to force their withdrawal from use in artificial insemination.

A study of the seasonal effects showed a highly significant decrease in percentage of motile spermatozoa during the early spring months and a highly significant difference between months in spermatozoa count. The lowest average count was found in August, but there was no significant difference in fertility of the bulls from month to month.

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RETENTION OF MOLD FRAGMENTS BY BUTTER, BUTTERMILK AND WASH WATER DURING MANUFACTURE OF BUTTER*

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In earlier studies on factors affecting mold content of cream and butter (5) it became apparent that more information was needed on retention of mold fragments by butter during churning and subsequent operations. The question of mold retention is of considerable importance since the validity of any mold fragment (mold mycelia) count on butter depends upon the percentage and uniformity of mold transfer from cream to butter during churning. If the percentage of mold carried over to butter were low, or more important, if the percentage carried over were quite inconsistent from one churning to another, a count of mold fragments in the butter could not provide a reasonable index of the mold content of the cream. If such were the case, use of the Wildman mold mycelia method as an index of mold content and quality of raw cream would be entirely unjustified.

Results of two previous investigations on this problem do not agree. Wildman (6) reported results of an experiment in which four samples of cream were churned and mold mycelia determinations run on buttermilk and butter. Mold mycelia counts on the butters were 76, 40, 92 and 68 and on the respective buttermilks 4, 4, 4 and 24. Naturally the results could not be quantitative because the Wildman method does not attempt to measure total mold filament in a sample. However, in one churning in which their yield was measured, 2 parts of butter to 3 of buttermilk were obtained. The butter had a mold mycelia count of 76 per cent and the buttermilk 4 per cent. A reconstituted sample consisting of 2 parts of the melted butter and 3 parts of the buttermilk showed a mold mycelia count of 60 per cent. Wildman concluded that churning actually concentrated the mold in the butter.

Adams and Parfitt (1) concluded that mold mycelia retention by butter of mold mycelia in cream was in general between 20 and 30 per cent. *Oospora lactis* strains were retained from 9 to 42 per cent with an average retention of 23 per cent. From the standpoint of dead mold fragment studies on butter, these results must necessarily be discounted because they are based on agar plate counts and no consideration was given to weights or volumes of butter, buttermilk and wash water involved.

In view of its significance and the disagreement between studies thus far on this subject, it seemed advisable to investigate it further.

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EXPERIMENTAL

Investigations were first carried out on laboratory churnings in quart mason jars. In order to more closely simulate the degree of agitation encountered in commercial churns, 1600-ml. quantities of sour cream containing varying amounts of mold were neutralized, pasteurized and then churned in gallon-size Dazey churns. The studies were then continued on commercial churnings. When this was done, a sample of the neutralized, pasteurized cream was collected as it went into the churn, and 1600 ml. of this sample also churned in a Dazey churn. Experimental lots of cream were churned at about 45° F. The temperature of these lots increased slightly during churning. The commercial lots were churned at about 48–50° F. About 25–30 minutes were required for the experimental churnings and 45 for the commercial. Some exceptions occurred and will be noted. A record of pounds or grams of cream, butter, buttermilk and wash water was kept for every churning. Because the amount of mold in the wash water was so small, only the first lot of wash water was analyzed for each of the commercial churnings. Only one washing was made in the case of laboratory churnings. In every churning an attempt was made to wash with a volume of water equivalent to that of the cream churned.

Estimation of total combined length of mold fragments in butter, buttermilk and wash water was made by the new quantitative method described in a previous paper (4). Some modifications in technique were necessary for buttermilk and wash water. Buttermilk was diluted at the rate of one gram per 19 ml. and wash water at the rate of 5 grams per 5 ml. hot gum solution. Cream was diluted in the same manner as butter. Total length of filament in all samples was calculated in terms of mm. per mgm., then total mm. of mold per gram or pound of sample calculated, and the total for the respective butter, buttermilk, wash water or cream in any one churning was determined. The percentage of the total visible mold filament contained in each portion of a churning was determined from the sum of that accounted for in butter, buttermilk and wash water. This procedure was considered preferable to using the cream count for the total because cream counts were less accurate than the others. Special methods of heating and agitating the cream samples are necessary to break up clumps of mold fragments.

It should be pointed out also that the counts included only visible mold fragments and not the tiny segments of *Oospora lactis* sometimes known as spores. In some trials attempts were made to include the tiny segments by using 500× magnification. They were significant in contributing to buttermilk and wash water, but not butter counts. However, the mold fragments that would contribute to an official mold mycelia count on butter were of primary interest and the studies therefore confined largely to fragments that might affect this value. Fragments 20 to 30 microns in length

were easily detected in butter and somewhat less readily in buttermilk. They were included in the counts. The tiniest fragments or so-called spores of *O. lactis* were about 10 microns in length.

Results in tables 1 and 2 indicate that in both laboratory and commercial churnings the mold fragments are predominantly retained by the butter. On a mgm. basis the total filament in butter usually ran several times greater than in buttermilk. Wash water generally contained much less mold than buttermilk. When weights of the different materials (butter, buttermilk and wash water) in a churning were taken into consideration, the butter in almost every case contained the majority of the mold that could be accounted for in all three materials. The total filament in butter, buttermilk and wash water usually approximated, roughly, the amount in the cream.

It was obvious that most of the longer mold filaments remained in the butter. There were many more long filaments in the butter than in the

TABLE 1

Total visible mold fragments retained by butter, buttermilk and wash water during laboratory churning

Churning	Sample	Total visible mold	Grams of sample	Per cent of total visible mold
		mm. per mgm.		
1	Butter	14.98	585	68.52
	Buttermilk	4.15	930	30.22
	Wash water	0.10	1560	1.26
2	Butter	28.92	400	64.14
	Buttermilk	5.71	1115	35.33
	Wash water	0.60	1560	0.53
3	Butter	154.58	375	79.50
	Buttermilk	11.69	1165	18.68
	Wash water	0.87	1535	1.82

other materials. The shortest filaments of about 10–50 microns in length were most numerous in the buttermilk although many were also observed in the butter. In some churnings where the low fat content or other factors increased the churning time and agitation considerably, it was observed that the filaments were for the most part shorter and retention in the butter was lower. The percentage of total mold in buttermilk and wash water was greater than usual in such cases. No. C–2 in table 2 is an example of such a churning.

The mold content of the cream does not seem to have affected appreciably the percentage retained in the butter, buttermilk or wash water, respectively. In table 1 the third churning contained many times as much mold filament as the others and yet the percentage retained in the butter is not significantly greater than in the first two churnings. Similar results may be noted in table 2. Controlled studies on this one phase might be

desirable. The length of filaments and amount of agitation during churning probably determine more than the mold content of the cream how much mold will be distributed between butter and the buttermilk in a churning.

There is some indication that a high fat cream results in more mold being retained in the butter than when a low fat cream is churned. This

TABLE 2

Total visible mold fragments retained by butter, buttermilk and wash water during commercial churning

Churning	Sample	Total visible mold	Grams of sample	Per cent of total visible mold
		mm. per mgm.	*(×454)*	
A–1	Butter	25.37	400	64.77
	Buttermilk	5.54	900	31.83
	Wash water	0.41	1300	3.40
B–1	Butter	13.25	635	92.42
	Buttermilk	.52	1265	7.23
	Wash water	.017	1900	0.35
C–1	Butter	15.24	945	81.82
	Buttermilk	1.56	1755	15.53
	Wash water	0.17	2700	2.65
B–2	Butter	15.33	822	72.91
	Buttermilk	3.46	1278	25.62
	Wash water	0.12	2100	1.47
C–2	Butter	10.74	504	48.95
	Buttermilk	3.46	1296	40.61
	Wash water	0.64	1800	10.44

may be related to churning time, amount of agitation required to bring about the reversal of phases and possibly other factors.

Judging from results in table 3 the Dazey churn provided about the same degree of agitation as the commercial churns since the total mold filament contents of butters obtained by the two methods were equivalent in every case.

TABLE 3

Total visible mold fragments in butter from laboratory and commercial churnings on same lots of cream

Churning	New quantitative method		Wildman method	
	Laboratory	Commercial	Laboratory	Commercial
	mm. per mgm.	*mm. per mgm.*	*per cent*	*per cent*
A	22.78	25.37	86	88
B–1	12.04	13.25	77	81
B–2	15.76	15.33	68	72
C–1	15.85	15.24	56	46
C–2	11.35	10.74	46	56

An interesting contrast is brought out by table 4. Percentage retention of bacteria differs greatly from that of the mold. The same slides as were

used for mold counts served also for bacterial counts. The oil immersion objective was used, however, for counting bacteria. The average number per field was multiplied by the microscopic factor to obtain the number per gram as for the microscopic count per ml. of milk (2). The percentages retained in the butter, buttermilk and wash water, respectively, were thus determined. The bacteria stained quite intensely and were easily recognized. Starter was added to the cream before churning lots C–1 and C–2 on which the bacterial study was made, and therefore sufficient bacteria were present to enable a fairly accurate study. That the bacterial counts were accurate is indicated by the fact that the total number accounted for in butter, wash water and buttermilk just about equaled the number in the cream before churning.

TABLE 4

Bacteria retained by butter, buttermilk and wash water during commercial churning

Churning	Sample	Direct microscopic count of bacteria	Grams of sample	Per cent of total number
		no. per gm.	(×*454*)	
C–1	Butter	25,980,000	955	1.15
	Buttermilk	1,065,180,000	1755	87.75
	Wash water	87,466,000	2700	11.09
C–2	Butter	8,660,000	504	0.75
	Buttermilk	415,680,000	1296	91.57
	Wash water	25,114,000	1800	7.68

The counts in every case indicated that bacterial cells tended to pass out into the buttermilk and also many more were removed by the wash water. An interesting fact in this connection was that the bacteria, which appeared to be a large-celled strain of *Streptococcus cremoris,* formed chains of cells which were in some cases longer than the filaments of mold retained by the butter. The chains of bacterial cells were about one-third to one-half the diameter (width) of the mold filaments.

DISCUSSION

The reason for the striking retention of mold filament by butter during churning is not entirely clear. Apparently the longer mold filaments are enmeshed or held by the fat phase when butter is formed during churning. These may be broken up to some extent during the ensuing working of the butter (3). It would seem that more of the long chains of bacterial cells might also be retained by the butter unless some difference in constitution, either physical or chemical, plays a part. Such explanations as effect of electric charge of bacteria and fat globules can only be speculative.

In evaluating the results, some consideration should be given to breaking up of filaments during churning and subsequent loss of the tiny fragments

in the buttermilk. This undoubtedly occurred to some degree and its extent could not be accurately determined by the counting methods used. Such a loss, if it could be determined, would actually lower the percentage figure retained for butter and increase that of the buttermilk. Studies with higher magnification indicated that when the shortest fragments were included, the retention figure for butter was lowered about 10–20 per cent and that of the buttermilk increased by this much. However, as mentioned earlier, these fragments would not contribute to the official mold mycelia count on butter and therefore were of less interest than the longer ones.

The results indicated that for studies of this nature the Dazey paddle churn provided conditions at least roughly approximating commercial churns. Since the total mold content of experimental and commercial butters from the same cream were about equal, the degree of agitation in the two types of churning must have been similar. This might not be true of churnings where quart mason jars are agitated to produce the butter.

For the most part the results substantiate the early report of Wildman regarding retention of mold by butter. It appears that most of the total mold filament is retained by the butter and that a high mold content cream is likely to produce a high mold content butter.

Nevertheless, such factors as fat content of cream, churning time and possibly others may be significant in affecting the carry-over of mold from cream to butter. The results indicate that in some churnings the variation in retention is enough to appreciably affect the mold mycelia count of butter. This is of significance in assessing the quality of butter on the basis of its mold mycelia count, particularly where rigid standards are enforced and butter consequently confiscated when it slightly exceeds the legal limit set by enforcement officials. Two lots of cream of equivalent mold content might not yield butters with the same mold mycelia count, if factors affecting retention enter into the picture. More data based on quantitative studies are needed on the significance of these factors.

SUMMARY

Total visible mold filament was determined on butter, buttermilk and wash water of both laboratory and commercial churnings.

Results indicated that butter usually retained more than 50 per cent of the total length of mold filament and that the wash water contained a very small percentage. The butter appeared to retain the long filaments during churning and most of the tiny fragments passed out into the buttermilk.

Studies on some commercial churnings indicated that butter retained a very small percentage of the bacteria of the original cream. Most of the bacteria were found in the buttermilk. This occurred in spite of the fact that many chains of the bacteria approximated the mold filaments in size.

The total mold content of laboratory churned butter approximated that of commercially churned from the same respective lots of cream.

The possible effects of other factors such as fat content of cream and degree of agitation during churning on retention of mold fragments by butter are briefly discussed.

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UTILIZATION OF UREA AND GROWTH OF HEIFER CALVES WITH CORN MOLASSES OR CANE MOLASSES AS THE ONLY READILY AVAILABLE CARBOHYDRATE IN THE RATION*

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In previously published studies on the utilization of urea by the ruminant, we presented data showing the need for a readily fermentable carbohydrate (4) in the ration, and for a low level of dietary protein (8), in order to have effective transformation of the urea nitrogen into protein. Corn starch was the carbohydrate studied. When urea was added to the basal ration of timothy hay only, hydrolysis of the urea to NH_3 and disappearance of the NH_3 from the rumen contents were very slow, and six hours after feeding there was no increase of the protein level of the rumen contents above that when the timothy hay alone was fed. When starch and urea were fed the protein level of the rumen contents rose rapidly and the ammonia nitrogen disappeared in six hours.

The question still remained whether a more soluble carbohydrate, such as that in molasses, would be as effective as starch in allowing utilization of the urea nitrogen. If it could so function it might be possible in sugar-producing countries to construct economically suitable rations for growing calves or milking cows from roughage plus urea and molasses, adequately fortified with bone meal, salt, and vitamin A if needed. The present experiment was set up with this purpose in mind. Corn molasses was used rather than cane molasses in order to provide a more rigorous test, since the corn molasses is practically free of nitrogen.

EXPERIMENTAL

For this study we used a 1,000-pound Holstein heifer with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed the experimental ration twice daily, at 8 A.M. and 6 P.M. Samples for analysis were taken from the rumen at intervals of 1, 3, and 6 hours after the morning feeding. Sampling was done twice weekly, on Monday and Friday, for a period sufficiently long to give constant results for several days. A period of three weeks after a change in ration was always allowed for an adjustment period before studies on the rumen con-

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tents were undertaken. The samples were taken by mixing the top solid matter adjacent to the fistula with the more liquid ingesta underneath until a uniform consistency was obtained, and then withdrawing a sample of about a kilogram. Four hundred grams of the sample were used for a dry matter determination, and 20-gram samples were used for triplicate determinations of urea, ammonia, and total nitrogen. The urea was determined by the urease method and the ammonia by magnesium oxide distillation. Urea nitrogen, ammonia nitrogen, and protein were calculated as per cent of the total dry matter present. The protein was calculated as (total nitrogen minus (NH_3–N plus urea–N) $\times$ 6.25).

The daily experimental rations in the order of feeding were as follows:

Period 1—Timothy hay alone (12 pounds). The hay for this and all subsequent periods was cut to lengths of $\frac{1}{2}$ inch or less by running through a hammer mill.

Period 2—Timothy hay (10 pounds) + corn molasses (4 pounds).

Period 3—Timothy hay (10 pounds) + corn molasses (4 pounds) + urea (200 grams).

Period 4—Timothy hay (10 pounds).

Period 5—Timothy hay (10 pounds) + commercial corn starch (2 pounds) + corn molasses (2 pounds).

Period 6—Timothy hay (10 pounds) + starch (2 pounds) + corn molasses (2 pounds) + urea (200 grams).

Period 7—Timothy hay (10 pounds) + starch (2 pounds) + corn molasses (2 pounds) + casein (0.4 pound).

Period 8—Same as period 7 + urea (200 grams).

Period 9—Timothy hay (10 pounds) + corn molasses (4 pounds) + urea (200 grams).

RESULTS

The results are summarized in table 1. The figures given are the average analyses of samplings on three or four separate days. The NH_3 + urea–N and total protein values are given as per cent of the dry weight for one, three and six hours after feeding.

The chief point to be observed is that the combination of timothy hay, molasses, and urea (Period 3) gave a protein level of the rumen contents of 9.5 to 10.0 per cent, somewhat lower than the level of 10.75–11.0 per cent protein obtained on feeding a similar ration in which starch had been substituted for one-half of the molasses (Period 6). In addition, the ammonia nitrogen disappeared more slowly on the hay, molasses, and urea ration than it did when starch was added, remaining at a level of 0.09 per cent at six hours, as contrasted with 0.024 per cent.

Results with the other rations, which acted as controls for the above two rations, were as expected, both the protein and NH_3–N remaining at low levels.

The addition of casein to the hay, starch and molasses (Period 7) raised the protein level of the ingesta to 9 per cent, as would be expected. The further addition of urea (Period 8) then caused the protein level to rise to 11 per cent of the total dry matter. The protein did not rise above this level, even though plenty of NH_3 was available, as shown by the fairly high NH_3–N at six hours. As previously shown (8), the effectiveness of urea utilization depends upon the level of preformed protein in the diet; the more protein above a certain level fed, the less urea will be utilized in raising the protein of the ingesta to the maximum.

When it was evident from the results just discussed that molasses was at least fairly effective in aiding urea utilization, it was decided to determine whether growing calves could meet their protein requirements largely from urea, with molasses as the carbohydrate source. Three heifer Holstein calves

TABLE 1

Influence of rations on urea utilization in rumen of fistula heifer
Per cent on dry basis

Period	Ration	NH_3–N + Urea–N			Total protein		
		1 hr.	3 hrs.	6 hrs.	1 hr.	3 hrs.	6 hrs.
1	Timothy hay	0.036	0.041	0.025	7.56	7.83	7.72
2	Timothy hay + molasses	0.009	0.010	0.008	6.58	6.17	6.52
3	Timothy hay + molasses + urea	0.139	0.153	0.090	9.79	9.98	9.28
4	Timothy hay	0.033	0.024	0.020	6.15	6.62	7.62
5	Timothy hay + starch + molasses	0.018	0.013	0.010	7.47	7.04	7.85
6	Timothy hay + starch + molasses + urea	0.158	0.150	0.024	11.06	10.77	10.90
7	Timothy hay + starch + molasses + casein	0.030	0.025	0.014	8.95	9.02	9.11
8	Timothy hay + starch + molasses + casein + urea	0.184	0.220	0.094	10.08	11.10	11.00
9	Timothy hay + molasses + urea	0.146	0.141	0.076	9.35	9.69	9.37

weighing about 200 pounds each were placed on a ration of the following composition:

Timothy hay	49
Cane molasses	60–70
Bone meal	2.0
Urea	2.5
Iodized salt	1.0
Shark liver oil capsules	

The total protein equivalent in the ration (nitrogen × 6.25) was 11.6 per cent. The timothy hay (6 per cent protein) supplied approximately 3 pounds and the cane molasses (1.6 per cent protein) from 0.96 to 1.02 pounds of protein per 100 pounds of ration. Seven to eight pounds of protein equivalent were supplied by the urea. Consequently the urea fur-

nished from 60 to 65 per cent of the total nitrogen in the ration. The cane molasses contained 71.1 per cent of solids and in these growth studies was used instead of corn molasses. The shark liver oil was furnished at a level supplying 1500 International Units per day per calf. The ration was thoroughly mixed except for the shark liver oil which was fed daily in capsules. The ration was a sticky, uninviting mixture, but in spite of this there was no failure of ready consumption. It was fed at an increased daily rate as determined by complete consumption. The calves were weighed weekly. Some milk was also fed for the first three weeks until the calves became accustomed to the ration. The molasses was increased from 60 to 70 parts after seven weeks when it became apparent that a maximum rate of growth was not being attained, possibly because the caloric intake was insufficient for their needs.

During a period of 19 weeks on the above ration the calves gained a total of 102, 104, and 82 pounds, respectively, or 0.77, 0.78, and 0.62 pounds per day, or about half the normal rate of growth. The weights of the calves after this period were 337, 290, and 267 pounds, respectively. See table 2 for summary of all of the growth data.

TABLE 2

Showing rates of daily gain on the different rations

Ration	Gains in wt. (lbs. daily)		
	No. 1	No. 2	No. 3
Timothy hay + cane molasses + urea + bone meal + iodized salt + vitamin A	0.77	0.78	0.62
Timothy hay + cane molasses + urea + 0.3 pounds casein + bone meal + iodized salt + vitamin A	1.7	1.5	1.6
Timothy hay + cane molasses + urea + 0.3 pounds starch + bone meal + iodized salt + vitamin A	1.3	1.4	1.5
Timothy hay + corn molasses + urea + starch + bone meal + iodized salt + vitamin A	1.4	1.5	2.0

It seemed evident that normal growth could not be obtained with such a ration as timothy hay, supplemented with molasses and urea. Since the intake of urea was large it was probable that the proteins of the microorganisms in the rumen were furnishing the larger share of the amino acids necessary for growth. We have other data involving urea feeding which show that a total protein equivalent in the ration of 9.49 per cent (2) was sufficient for growth in calves at a rate of 1.0–1.2 pounds per day. *Such rations always contained a certain amount of natural protein derived from yellow corn.* Consequently we were inclined to believe that the proteins produced on the ration used in these later experiments were more likely deficient in quality than in quantity of total protein. With this point in view, we added 0.3 pound of casein per day (commercial crude) to the ration of each calf in order to determine whether the slow growth was due to a

protein deficiency. This addition raised the total protein equivalent in the ration to 13.5 per cent on the basis of a daily consumption of 14 pounds of the basal ration.

Over a period of seven weeks No. 1 gained 83 pounds (daily average 1.7 pounds), No. 2 gained 72 pounds (daily average 1.5 pounds), and No. 3 gained 78 pounds (daily average 1.6 pounds). These results indicate that the molasses, urea, and timothy hay mixture was either not able through the proteins of microorganisms to provide protein of best quality for optimum growth or to provide sufficient protein. While the total protein equivalent of the ration without casein was 11.6 per cent, comparing well with that of successful rations made of urea, starch, *corn meal* and timothy hay, it must be kept in mind that in an open system such as prevails in the ruminant's tract, some of the molasses sugar may have moved out of the rumen faster than was the case with starch. This would account for the somewhat lower protein in the rumen content and explain why an addition of 0.3 pound of casein daily increased markedly the rate of growth.

After seven weeks on the ration containing casein the latter was withdrawn and in its place 0.3 pound of corn starch was substituted daily. Because of the shortage of cane molasses it was only possible to make growth observations over a span of 4 weeks. In this period No. 1 gained 36 pounds, equivalent to 1.3 pounds per day. No. 2 gained 40 pounds, equivalent to 1.4 pounds per day. No. 3 gained 41 pounds, equivalent to 1.5 pounds per day. The average daily gain of the three calves in this period was 1.4 pounds as compared with 1.6 pounds daily during the period of casein feeding, but it was a much better rate than secured on the molasses ration alone and compared favorably with the gains on the molasses-casein ration. Evidently the addition of the small quantity of starch had allowed a more efficient protein manufacture to proceed—probably due to the continuous hydrolysis of the starch and a more continuous source of soluble sugar.

To test further the hypothesis that with carbohydrate and urea an effective protein mixture for normal growth could be secured, the three calves were changed to the following ration:

42.25	pounds timothy hay
32.75	pounds starch
20	pounds corn molasses
2	pounds bone meal
2.5	pounds urea
1	pound iodized salt

Vitamin A was furnished as shark liver oil in gelatin capsules. No other protein source was available except that of the timothy hay. Corn molasses was used to improve the palatability and also to introduce a sugar containing no nitrogen. On this ration No. 1 gained 81 pounds in 8 weeks equivalent to 1.4 pounds per day. No. 2 gained 82 pounds in 8 weeks equivalent

to 1.5 pounds per day. No. 3 gained 114 pounds in 8 weeks equivalent to 2.0 pounds per day.

These are considered normal rates of gain for this species. It is evident that a ration carrying, in addition to the roughage, only molasses with a small amount of starch or the reverse can adequately function with urea as the main source of nitrogen. Further, when rumen conditions are optimal for the growth of the microorganisms the amount and quality of the proteins produced can cause a maximum rate of growth of heifer calves.

DISCUSSION

From the results of the first phase of the feeding experiments it seemed possible that not enough protein was formed in the rumen and what was formed may have been of poor quality. As stated above, it has been shown (2) that calves will grow almost normally on rations where the only nitrogen sources were yellow corn, starch, timothy hay, and urea, and at a protein equivalent level of 9.49 per cent. This seemed to indicate that the protein formed from the urea by the microorganisms in the rumen was of fair quality but that the corn proteins may have acted as effective supplements. In the case of lambs, Harris and Mitchell (1) reported that the addition of urea to a ration unable to support appreciable growth converted the ration into one capable of promoting a nearly normal rate of growth. In all of our work on growth or milk production with calves or cows the greatest effectiveness of urea was obtained only in the presence of certain grain proteins and starch.

The experiments outlined in this paper would indicate that proteins of high quality and sufficient quantity can be formed in the rumen by the microorganisms there, provided some slowly hydrolyzable carbohydrate is provided, such as starch. A roughage plus urea and a soluble sugar alone will be less effective. No doubt our early success in attaining normal growth of calves with a ration containing corn meal and starch was mainly due to the provision of a slowly hydrolyzable carbohydrate such as the starch in the corn meal or starch itself.

These results also raise the question as to the validity of the idea that the biological importance of protein is of less significance in the case of the ruminant (7) than with other animals. At the moment the idea prevails that the ingested proteins in the case of the ruminant are largely converted to microorganism proteins and these then serve as the primary amino acid source for the animal. This idea bans the older conception that liquids and finely ground feeds largely pass directly through the esophagal groove into the lower digestive tract.

The experiments outlined in this paper contribute nothing definite toward an answer to that problem. However in 1915 (3) Hart and Humphrey published data showing that for maintenance and milk production in

cows the proteins of milk showed a definitely greater efficiency than those of corn or wheat. Positive nitrogen balances were maintained with milk proteins for the production of as high as 35 pounds of milk per day but negative balances resulted when the proteins at the same level were derived from the corn or wheat grain. Such results indicate that all ingested protein is not reworked to the protein of the microorganism but that at least some of that in finely divided condition may go directly through the rumen to the lower digestive regions.

The results of the fistula experiments indicate that only partial utilization of urea by ruminants occurs when molasses is the chief source of readily fermentable carbohydrate. Protein is formed in the rumen, but the final level of protein reached is not as high as when a less soluble carbohydrate is in the ration. This does not mean that the combination of urea and molasses is ineffective, but that it is not as efficient as a combination of urea and starch. The calf growth experiment indicates the same fact. These results agree with those of Pearson and Smith (6), who found that upon the incubation of rumen ingesta *in vitro* with urea and various carbohydrates, starch was most effective in causing synthesis of protein, as indicated by the decrease in NPN in the medium. Galactose and maltose were also good, sucrose was fair, while dextrin, glucose, glycerol and lactic acid were relatively poor.

In another paper of the same series (5), they conclude that *in vivo* experiments of the type reported here cannot be expected to yield any certain evidence of protein synthesis from urea until truly representative samples of total ingesta can be obtained and analyzed, and until more is known of the effect of urea on the passage of the various dietary constituents through the rumen. We agree that obtaining representative samples of the entire rumen is very difficult, and that little is known of the rate of passage of various constituents out of the rumen. However, we believe that reliable results can and have been obtained with our technique in spite of these difficulties. Instead of trying to obtain samples representative of the entire rumen content, we took samples from the same spot every time, made as similar as possible by controlling the moisture content. By this method samples can be taken from day to day that vary only slightly in percentage composition; triplicate determinations on the same sample check very closely.

SUMMARY

1. Further studies on urea utilization in a rumen fistula heifer are reported.

2. With timothy hay as the sole ingredient of the basal ration utilization of urea was low. Corn molasses provided a suitable substrate for the development of an active flora, and urea was fairly well utilized. The

protein of the rumen contents (dry basis) rose from a basal level of 7.7 per cent to 9.28 per cent.

3. With timothy hay, starch, corn molasses and urea as the ration the protein level in the rumen contents rose from 7.7 per cent to 10.9 per cent. Apparently somewhat better utilization of urea was made on a starch-containing ration than on one containing mainly a more soluble sugar.

4. In growth experiments with young heifer calves a ration made of timothy hay, *cane* molasses and urea fortified with common salt, bone meal, and vitamin A gave a subnormal rate of growth (0.6 to 0.8 pound daily).

5. When this ration was supplemented with 0.3 pound of crude casein daily normal growth was attained. Substitution of an equal weight of corn starch for the casein likewise resulted in normal growth.

6. For maximum growth of calves a ration made of a roughage, molasses and urea must be supplemented with some additional source of a more insoluble but fermentable carbohydrate or insoluble protein which can be drawn from the cereal grains or concentrates, such as the oil meals. In our limited experience this supplemental material need not be more than 3–5 per cent of the total ration.

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RUMEN SYNTHESIS OF THE VITAMIN B COMPLEX AS INFLUENCED BY RATION COMPOSITION*

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Since Bechdel *et al.* (2) reported that cattle, and possibly all other ruminants, possess the ability to synthesize the vitamin B (complex) in the rumen, workers have been interested in the problem from the following points of view. First, what effect do the constituents of the ration have upon the synthetic powers of the organisms involved and the quantity of vitamin produced; second, what types of organisms are responsible for these syntheses. Wegner *et al.* (21) reported that cows are able to synthesize thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, and biotin; and further, the addition of thiamine to the ration apparently increases the synthesis of the above members of the B-complex except nicotinic acid. They (22) also found that increased nitrogen as added casein in the ration did not increase synthesis; in fact it decreased it in the case of riboflavin. McElroy and Goss (10, 11, 12, 13, 14, 15), working on the same problem, reported that sheep and cows were able to synthesize riboflavin, pyridoxine, thiamine and pantothenic acid on vitamin-low rations. Jukes and McElroy (15) have also shown that biotin was synthesized in the rumen. Hunt *et al.* (4, 5) reported that there was a direct correlation between riboflavin synthesis and the carbohydrate in the ration, and that *ground* yellow corn was better than whole corn as a substrate in the synthesis of riboflavin. They were unable to detect any thiamine synthesis in their experiments as judged by the comparative amounts in the ration and the rumen at various time intervals after feeding. On an exclusive alfalfa hay ration they found less riboflavin in the dried ingesta of the rumen than in the hay; suggesting either that the riboflavin was rapidly absorbed by the animal or in part destroyed. Kick and associates (7) reported that the rumen contents of steers fed alfalfa hay exclusively were alkaline in reaction, whereas if grain and a protein supplement were fed with alfalfa hay the rumen contents were acid in reaction. These results would in part explain the low values reported above for riboflavin since it is destroyed in an alkaline medium. The normal pH of the rumen will vary with the ration, but is usually between 6.8–7.3. McElroy and Goss (12) reported that adjusting the pH of the rumen samples to

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4.5–5.0 with concentrated HCl before drying enhanced the riboflavin values. Wegner *et al.* (22) reported that adjusting the pH to 4.5–5.0 did not increase the riboflavin values as was reported by McElroy and Goss.

In this paper there is reported the effect of nitrogen added to the ration as urea and the extent of the vitamin B-complex synthesis in the rumen of the fistulated cow and calf. The aim was to determine if there was any correlation between added nitrogen and carbohydrate on the vitamin synthesis in the rumen.

EXPERIMENTAL

The animals were fed the following rations for a period of one month.

Ration

1. Timothy hay (12 lbs.)
2. Timothy hay (10 lbs.) Corn molasses (4 lbs.)
3. Same as No. 2 plus 200 grams of urea per day.
4. Timothy hay (10 lbs.)
5. Timothy hay (10 lbs.) Corn molasses (2 lbs.) Starch (2 lbs.)
6. Same as No. 5 plus 200 grams of urea per day.
7. Timothy hay (10 lbs.) Corn molasses (2 lbs.) Starch (2 lbs.) Casein (acid washed) (0.2 lbs.)
8. Same as No. 7 plus 200 grams of urea per day.

At the end of each period a sample of the rumen content was removed for vitamin assay. The samples were very constant in composition on the basis of moisture content. The sample and an amount of 95 per cent ethyl alcohol, equal in volume to the weight of the sample, were placed in the cold room, 7° C., for one week to stop bacterial action and fermentation. The contents were then placed in enamel trays and dried at 37–40° C. The dried samples were ground in the Wiley mill and assayed for biotin (16), nicotinic acid (8, 18), pantothenic acid (20), riboflavin (17, 19), thiamine (3), folic acid[1] (9), and pyridoxine (1). All of the constituents of the ration were assayed for the above vitamins. (See tables 1, 2 and 3.)

RESULTS

The addition of nitrogen, as urea, resulted in increased synthesis of nicotinic acid, biotin, riboflavin and pantothenic acid in the rumen, but significantly only when molasses or a readily fermentable carbohydrate was supplied with the ration.

The synthesis of pyridoxine could not always be correlated with the variation in ration composition. However, in some instances a correlation was obtained, as for example, with rations 3 and 6. In these instances the addition of urea to the ration containing a fermentable carbohydrate definitely increased the synthesis of pyridoxine.

[1] The folic acid values are calculated as follows:

$$\frac{\%\ \text{activity}}{4} = \gamma/\text{gram}$$

There was no direct correlation between increased urea intake and vitamin synthesis in respect to "folic acid."

TABLE 1

Cow—vitamin content per gram of dry rumen material

Ration	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid	Biotin	Folic acid	Pyridoxine
	μg./gram	μg./gram	μg./gram	μg./gram	mμg./gram	μg./gram	μg./gram
1	0.17	6.7	31.0	3.4	207	0.42	2.6
2	0.24	7.6	39.6	3.9	253	0.33	2.6
3	0.36	13.6	64.7	12.0	277	0.42	4.2
4	0.18	7.7	35.2	5.8	207	0.52	2.6
5	0.18	5.3	30.9	5.7	212	0.31	2.5
6	0.08	12.7	65.6	17.9	289	0.57	4.3
7	0.12	11.5	56.6	18.2	250	0.34	2.8
8	0.35	12.0	59.0	16.3	309	0.49	2.6

TABLE 2

Calf—vitamin content per gram of dry rumen material

Ration	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid	Biotin	Folic acid	Pyridoxine
	μg./gram	μg./gram	μg./gram	μg./gram	mμg./gram	μg./gram	μg./gram
1	0.39	7.4	24.3	5.3	210	0.41	3.2
2	0.14	5.2	31.0	5.2	224	0.33	2.0
3	0.46	10.3	49.6	10.8	299	0.37	3.9
4	0.69	9.7	40.4	9.5	188	0.64	3.3
5	0.45	8.6	40.4	5.8	200	0.24	2.7
6	1.00	15.7	57.2	13.3	307	0.33	3.6
7	0.59	11.6	56.8	17.8	240	0.55	2.8
8	0.65	12.8	70.8	18.0	294	0.49	2.8

TABLE 3

Vitamin content of ration ingredients per gram of dry material

Material	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid	Biotin	Folic acid	Pyridoxine
	μg./gram	μg./gram	μg./gram	μg./gram	mμg./gram	μg./gram	μg./gram
Timothy hay	1.8	5.5	23.5	8.6	62.2	.....	3.9
Corn molasses	< 0.1	< 0.05	< 0.05	< 0.05	< 10.0	< 0.1	< 0.1
Corn starch	< 0.1	< 0.05	< 0.05	< 0.05	< 15.0	< 0.1	< 0.1
Urea	< 0.1	< 0.05	< 0.05	< 0.05	< 12.0	< 0.1	< 0.1
Casein—acid washed	< 0.1	< 0.05	< 0.05	< 0.05	< 20.0	< 0.05	< 0.05

In the case of thiamine and with a non-synthetic type of ration we encountered the same effect as observed by Hunt *et al.* (4, 5). The value

obtained indicate that there was little, if any, synthesis of this essential vitamin. It was reported by Kennersley *et al.* (6) that thiamine is oxidized in alcoholic solution if allowed to stand for several months. To test this point four samples of rumen contents were dried directly, omitting the alcoholic storage, and the same values as shown above were obtained, indicating no destruction by storage in alcohol.

Timothy hay is the only constituent of the ration that contained any appreciable amount of the above vitamins (table 3).

SUMMARY

The addition of urea as a source of nitrogen definitely increased the synthesis of riboflavin, nicotinic acid, biotin and pantothenic acid in the bovine rumen when a readily available carbohydrate was present. Pyridoxine and "folic acid" could not be too closely correlated with ration composition. In the absence of a readily fermentable carbohydrate and probably a low population of microorganisms the synthesis of the members of the B complex is not at a maximum.

The data indicate that thiamine may not be synthesized in the rumen. However, it seems more than probable that it is synthesized, but absorbed or destroyed at a rate greater than its synthesis.

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CAROTENE LOSSES IN FRESHLY CUT PLANT TISSUES*

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The carotene in hays and silages is the most important source of vitamin A for dairy cows. The amount of carotene in hays is largely dependent upon the curing process. Hays may contain relatively small amounts of carotene even though they were made from fresh, green plant materials with high initial carotene contents. In studying this loss of carotene during the hay-curing process Hauge (8) obtained evidence for the presence of a carotene-destroying enzyme in alfalfa. That there may be differences in enzyme activity in different plants is indicated by the work of Bolin (3) who found that when fresh plant material was stored at five degrees F. for ten months, the loss of carotene in alfalfa was 62.7 per cent while there was little or no loss in bromegrass, meadow fescue, orchard grass, and Kentucky blue grass. During the summer of 1941 the authors found that bluegrass, even though dry and brown in color due to lack of rainfall, contained a surprisingly large amount of carotene. This suggested the possibility that bluegrass might be low in the carotene-destroying enzyme. In studies with grasses and berseem, Seshan and Shen (12) concluded that neither mold or bacterial action affected carotene losses and apparently doubted that enzymes have any great effect upon carotene losses in these plants. With these facts as a basis, it seemed desirable to study the losses of carotene in various plant materials under conditions which were favorable to enzyme activity and under other conditions which inhibited enzyme actions.

EXPERIMENTAL

The relative enzyme activity of various plant materials was determined by the loss of carotene during an incubation period at a favorable temperature. The difference in loss of carotene between two samples, the enzymes having been inactivated by heat in one and not in the other, may be attributed directly to the effect of the enzymes. Some samples were incubated in an atmosphere of nitrogen. Any decreases in carotene losses of autoclaved samples incubated in an atmosphere of nitrogen are probably due to inhibition of uncatalyzed oxidation. Any decreases in carotene losses of samples not autoclaved but incubated in an atmosphere of nitrogen should be due to inhibition of uncatalyzed oxidation and also to inhibition of catalyzed oxidation if the enzyme is aerobic.

The plant materials, which had been secured fresh from the field, were immediately chopped into quarter to half-inch lengths and mixed. In the

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first series of experiments weighed samples were placed into a series of stoppered test tubes and treated as follows:

1. Fresh material, no treatment.
2. Incubated 24 hours at 37° C. in a hot air oven.
3. Autoclaved five minutes in steam at 115° C., followed by incubation for 24 hours at 37° C.
4. Incubated 24 hours at 37° C. in an atmosphere of nitrogen.
5. Autoclaved five minutes at 115° C., followed by incubation for 24 hours at 37° C. in an atmosphere of nitrogen.
6. Autoclaved five minutes at 115° C.
7. Dried in vacuum oven at 100° C. for 24 hours.

The carotene content of these samples was determined by a modification of Moore's method (9). After the carotene had been transferred from the alcohol-petroleum ether solution into petroleum ether, the solution was saponified by shaking in a separatory funnel for about one minute with 25 ml. of 25 per cent potassium hydroxide in methyl alcohol. This was followed by washing with water until free of alkali and alcohol, drying with anhydrous sodium sulfate, and passing the petroleum ether carotene solution through a dicalcium phosphate column as described by Moore.

The results of these experiments are shown in table 1. It was found that the loss of carotene upon incubation of the fresh material was greatest in alfalfa and red clover and lowest in oat, bluegrass, and bromegrass. However, in the samples which were autoclaved before incubation a high percentage of the carotene was retained in all cases. The differences in losses between the two treatments indicated that the chief losses during treatment 2 were due to the enzyme activity in the plants, while the variation in losses indicates considerable variation in enzyme activity of the different plant materials studied.

Inactivation of enzymes by autoclaving or vacuum drying did not greatly affect the carotene content. This shows that the carotene in plant materials is fairly stable to these heat treatments.

Samples incubated in an atmosphere of nitrogen retained a high percentage of their carotene. This indicates that the enzyme is probably aerobic and may be the same as the one described by Haas and Bohn (7) and studied by several other workers (1, 2, 5, 6, 10, 13, 18). Further evidence of this relationship was found when aqueous extracts of alfalfa, red clover and bluegrass were tested for carotene-destroying enzymes by the method of Reiser and Fraps (10). It was observed that the destruction of carotene in these solutions was similar to that observed in the plants. This indicates that the carotene-destroying systems in the different plants are similar.

Autoclaved samples lost some carotene upon incubation which indicates that non-enzymic oxidation occurred. This may explain why some of the

TABLE 1

Micrograms of carotene per gram of plant material (dry basis) after the indicated treatments to show variations in the content of carotene destroying enzymes

Date 1943	Material	*Treatment of sample							Remarks
		1 (*F)	2 (I)	3 (A, I)	4 (I, N)	5 (A, I, N)	6 (A)	7 (VD)	
9–27	Alfalfa-leaves and stems	454.3	88.2	321.6	210.5	401.4	487.6	411.4	Height—12″
6–21	Red clover-leaves	491.3	127.9	405.5	464.5	525.3	508.3	.....	First growth
7–1	Seresia lespedeza-leaves	281.3	171.5	239.6	250.1	268.5	273.2	259.5	Height—2′
7–15	Swiss chard	465.4	216.6	415.3	457.9	451.9	385.5	411.6	Height—12″
7–5	Corn-leaves	563.6	248.2	435.8	494.1	447.0	465.9	447.4	Height—4′
6–15	Oat	387.8	317.1	333.9	360.6	362.6	373.2	340.4	Height—8″
7–19	Bluegrass	528.9	405.6	462.0	486.6	498.3	540.9	474.9	Clippings
6–24	Bromegrass	726.0	558.1	624.0	652.9	668.4	621.6	646.6	Height—6″

* F—fresh.
I—incubated 24 hours at 37° C.
A—autoclaved 5 minutes at 115° C.
N—air replaced by N.
VD—vacuum dried 24 hours at 100° C.

samples incubated under nitrogen were higher in carotene than those incubated in air following autoclaving.

In order to eliminate any effect that the process of autoclaving might have upon carotene losses, a series of experiments was conducted in which samples were both incubated and autoclaved. One sample of the material was autoclaved and then incubated, and the other was incubated and then autoclaved. Thus each sample received the same treatment some time in the procedure and consequently any differences in the carotene contents may be ascribed solely to the effect of the enzyme. The results of these experiments are shown in table 2. It again becomes evident that there is considerable variation in the enzyme activity of plants.

TABLE 2

Micrograms of carotene per gram of plant material (dry basis) after incubation with and without enzyme inactivation

Date 1943	Material	Treatment		Per cent carotene destroyed	Remarks
		*AI	IA		
7–22	Corn-leaves	519.8	264.9	49.0	Height—3′
7–22	Ladino clover-leaves	104.0	29.8	71.4	Second growth
8–6	1st year sweet clover	86.0	12.9	85.0	Height—1′
8–6	Korean lespedeza-stem and leaves	263.5	212.0	19.5	Height—4″
8–9	Soybean-leaves	432.8	368.6	14.8	Bloom stage
8–9	Bluegrass	548.8	439.0	20.0	Clippings
8–11	Timothy	245.9	150.9	38.7	First year growth Height—8″
9–7	Corn-leaves	381.0	252.2	33.8	Early dent stage
9–7	Korean lespedeza-stem and leaves	219.0	201.9	7.8	Height—6″
9–3	Soybean-leaves	315.0	188.2	40.1	Pod stage

* AI—Autoclaved 5 minutes at 115° C. followed by incubating 24 hours at 37° C.
IA—Incubated and then autoclaved.

These experiments verify the earlier observations of Hauge (8), who concluded that enzyme action was responsible for a considerable portion of the large initial loss of carotene in alfalfa which follows the cutting of the plant.

The data presented indicate that certain plant materials, because of their low enzyme activity, should lend themselves more readily than others in grass mixtures for the production of hays and silages of high carotene content. Undoubtedly there is considerable enzymatic destruction of carotene during the wilting of some plant materials before ensiling and therefore reduction of the interval of time between cutting and ensiling should result in silage of higher carotene content. Russel *et al.* (11) found that in the field-curing of hay the carotene losses were greatest immediately following cutting and the rate of losses was closely correlated with conditions favorable to enzyme action. Dehydration of the plant material is one of the factors

slowing the enzyme action. Camburn *et al.* (4) found greater percentage loss of carotene in sun-cured than artificially dried hays during storage. This indicates that the enzymes are active in dry stored materials. If this is true, the advantage of inactivating the enzyme or having material with low enzyme content is apparent.

Further investigations should be made to study factors which affect the enzymatic activities of plant materials to obtain information that would be helpful in conserving the carotene in hay, silage and certain dehydration products.

SUMMARY

Studies have been made to determine the losses of carotene in freshly cut plant materials under conditions which were favorable to enzyme activity and under other conditions which inhibited enzyme action.

Evidence is presented which indicates that the destruction of carotene, due to enzymatic activity, is greater in alfalfa, red clover, and sweet clover than in the oat plant, Kentucky bluegrass and bromegrass. Other plants such as corn, soybeans, and lespedeza seem to have an intermediate enzyme activity. The enzyme appears to be aerobic in character.

Although the carotene losses in wilted plant materials are related to enzyme activity, some non-enzymic destruction also occurs.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

223. Biochemistry of the Fatty Acids—And Their Compounds, the Lipids. W. R. BLOOR, Prof. of Biochemistry and Pharmacology, Univ. of Rochester, Rochester, N. Y. Reinhold Publishing Co., New York, 1944.

The author has made a distinct contribution to the biochemical field in this monograph because he has brought together in one volume the present information concerning the fatty acids and their related compounds. The organization of the material is well carried out. Chapter I. Chemistry: Descriptive and Analytical, covers Classification of Lipids, Physico-Chemical Conceptions of the Lipids, and Macro and Micro Methods under Methods of Examination of Tissue Lipids. Chapter II. Digestion and Absorption, comprises Nutritional Availability of Fats, Lipid-splitting Enzymes, Fat Digestion, Absorption of Fats, and Digestion and Absorption of Other Lipids. Chapter III. Lipids of the Blood, contains such items as Normal Basal Levels of Blood Lipids, Changes in the Post-absorptive Level Produced by Food, Variations in Blood Lipids in Normal Lipids, Effect of Abnormal Conditions on the Blood Lipids, Infections, Mental Disease, Organic Diseases, and Effect of the Lipids of the Blood on its Properties. Chapter IV. The Lipids in Tissue, lists Data on Tissue Lipids, Tissue Lipids in Abnormal Conditions, Nature and Function of Tissue Lipids, Lipids of Plants, and Lipid of Microorganisms. Chapter V. Lipid Metabolism, is devoted to metabolic and catabolic features, such as Physiological Synthesis of the Lipids, Intermediary Metabolism and the Role of the Liver, Catabolism of the Fats, Fat Metabolism in the Developing Embryo, and the Vitamins in Lipid Metabolism. The last chapter, Chapter VI. The Lipids of Secretions and Excretions, takes up under Food for the Embryo and Young Organism, The Placenta, Milk and Eggs. In addition Lymph and Chyle, Cerebrospinal Fluid and Bile are considered, and finally Excretions. Each chapter is followed by an extensive bibliography varying in length from about three to seven and one-half pages. L.M.D.

BACTERIOLOGY

224. The Burri Slant Technique in the Food Industries. C. K. JOHNS, Dept. of Agr., Ottawa. Food in Canada, *4*, No. 3: 17. 1944.

A test first described by Burri in 1928 can be often used to examine dairy products and other foods, in place of more expensive and complicated methods. The medium recommended is tryptone glucose skim milk agar

with brom-cresol purple added. The use of an additional 0.5% agar is suggested and an oval rather than a round tube may be preferable. The size of the inoculating loop may be varied to accommodate the number of organisms. O.R.I.

225. **Few Bacteria in Canadian Eggs.** C. K. JOHNS, Dept. of Agr., Ottawa. Food in Canada, *4*, No. 4: 16. 1944.

This author had previously reported on the numbers of bacteria found in storage eggs. The present study was done on fresh eggs and indicates that low counts are commonly found. Of 219 eggs examined, 77% had counts of less than 100 organisms per gram. O.R.I.

CHEMISTRY

226. **The Antimony Trichloride Method for the Determination of Vitamin A.** G. H. BENHAM, McGill Univ., Montreal. Canad. Jour. Res., *B. 22*, No. 2: 21. 1944.

A critical description of the antimony trichloride method for the determination of vitamin A is presented. Low values for vitamin A result from:

(1) Incomplete extraction from the alcoholic soap solution by using petroleum ether instead of ethyl ether.

(2) Incomplete separation of the layers during extraction and washing.

(3) Incomplete filtration through anhydrous sodium sulfate.

If strict attention is paid to details of procedure, the method gives consistent results. Uncertainty in regard to the exact factor for converting the *E* values to international units makes it impossible at this time to state accurately the absolute values. It is pointed out that this in no way detracts from the usefulness of the chemical test. O.R.I.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

227. **Concentrated Dairy Products.** J. L. PERLMAN AND A. H. ROBERTSON, Dept. of Agr. and Mkts., Albany, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 43, 1943.

The use of neutralizers to conceal inferior quality products in concentrated dairy products is an old practice that is deplorable. Neutralizers are used alone and in combination with sirups, stabilizers, flavors, etc. Special consideration is given to frozen desserts. Methods of detecting neutralizers are not simple and one of the best known is the Hillig method based upon the alkalinity of the ash. Data are presented on frozen desserts to show that this method detects as little as 0.05% sodium bicarbonate in milk and 0.1% in heavy cream. A.C.D.

DISEASE

228. Bovine Brucellosis. ANONYMOUS. Univ. of Ill., Col. of Agr. Cir. 573. 4 pages. March, 1944.

This leaflet lists nineteen "dont's" to be observed in developing and maintaining a milking herd free from Brucellosis (Bang's disease).

J.G.A.

229. Vitamin-D Deficiency in Dairy Cows. G. C. WALLIS. S. Dak. Agr. Expt. Sta. Bul. 372. 16 pages. March, 1944.

Symptoms, causes, and treatment are outlined in some detail.

How much vitamin-D deficiency there is in dairy cattle is not known. Cows on pasture during the summer ordinarily build up vitamin-D reserves due to exposure to the direct rays of the sun. When they are then fed during the winter on sun-cured roughages high in vitamin D, they probably receive more than 12,000 to 15,000 International Units of vitamin D daily. These amounts are about enough, according to studies by the author. However, even then milk production, calving records, and general health might be improved during the winter by some additional vitamin D.

On the other hand, there are undoubtedly many times when roughages low in vitamin D are fed. Then when spring comes, cases of mild vitamin-D deficiency and lowered general health and producing ability may occur.

Further information is needed to determine the vitamin-D content of various roughages and the degree of vitamin-D deficiency that may exist in dairy cows in winter. Until this information is available, a farmer will do well to be sure that his cattle get generous amounts of sun-cured hay and that they are exposed to sunshine often, particularly during the summer.

J.G.A.

230. A Bromthymol Blue Field Test for Bovine Mastitis. FRANCIS J. HALLINAN, N. Y. State Dept. Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 121, 1943.

The usual procedure for detecting mastitis by the bromthymol blue method is subject to certain errors that can be corrected. The author presents a simple procedure for field test using reproducible permanent standards prepared from Munsell color papers. A.C.D.

231. Augmenting War-time Milk Production by Converting Cows Condemned for Mastitis to Useful Three Teaters. F. W. GRAVES, N. Y. State Dept. of Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 11, 1943.

Under war-time conditions much milk is lost unnecessarily by condemnation of three-teated cows. If such cows can be saved, there will be a better

opportunity to cull unprofitable cows. The Federal Meat Inspection Service does not condemn the entire carcass because one area is infected; the same policy can hold true for milk inspection.

If a quarter is infected, it should be dried up. If it dries up well it may never milk again. There is also the chance that it may give good milk when the cow freshens. If completely dry the three-teater is a safe cow. If the infected quarter discharges milk it should be treated by a veterinarian to dry it permanently. This is done by passing the fumes of 4 ounces of ether directly into the infected quarter and after one month removing such material as can be withdrawn by milking. The quarter will be permanently dry. A.C.D.

FEEDS AND FEEDING

232. Single Grains and Grain Mixtures as Supplements to Alfalfa Hay and Silage for Milk Production. J. R. DAWSON, A. L. WATT, C. W. MCINTYRE, R. E. LEIGHTON, AND R. R. GRAVES. U. S. Dept. Agr. Cir. 696. 11 pages. Feb., 1944.

At each of three field experiment stations, two groups of four cows each were fed unlimited quantities of alfalfa hay and silage throughout their lactation periods of 365 days. In addition to this basic ration of roughage, one group of cows was fed a single grain—ground barley, ground corn, or ground kafir—at the rate of approximately 1 pound to each 6 pounds of milk they produced. The other group was fed a grain mixture, at approximately the same rate, which consisted of four to six different grains, grain by-products, and high-protein concentrates.

The cows receiving the single-grain ration had somewhat better breeding records during the experiment, gained more weight, and produced 95% as much butterfat as the cows on the mixed-grain rations. However, the results were not consistent and the difference in production therefore is probably not significant.

This experiment does not indicate the relative value of a single-grain ration and a mixed-grain ration when the roughages are so restricted that a large proportion of the nutrients must be furnished by the grain part of the ration. It does show that where cows have an opportunity to consume as much good hay and silage as they like, it makes little difference whether the additional nutrients they require are obtained from a single grain or a mixture of several grains and grain by-products.

At current market prices the cost of the feed required to produce a pound of butterfat averaged slightly less for the single-grain rations than for the mixed-grain rations. J.G.A.

233. Carotene Requirements for the Maintenance of a Normal Spinal Fluid Pressure in Dairy Calves. L. A. MOORE, M. H. BERRY, AND

J. F. SYKES, Dairy Dept., Univ. Maryland, College Park, and Dept. of Physiol., Mich. State Col., East Lansing. Jour. Nutr., *26*, No. 6: 649–658. Dec., 1943.

Two groups of calves (Holstein and Ayrshire) were used to study the amount of carotene required to maintain a normal spinal fluid pressure. One group was located at East Lansing, Michigan, and the other at College Park, Maryland. Alfalfa meal was used as a source of the carotene supplement. Ophthalmoscopic observations were made periodically. Blood plasma vitamin A and ascorbic acid values were also determined.

An intake of 66 micrograms per kilogram of body weight during the winter months is about the minimum requirement for carotene for Holstein and Ayrshire calves when spinal fluid pressure is used as a criterion. Because of individual characteristics, plasma vitamin A or carotene values will not distinguish between variations of carotene intakes of 62 to 75 micrograms per kilogram of body weight. C.F.H.

234. The Use of Urea in Making Silage from Sweet Sorghum. A. E. CULLISON, Miss. Agr. Expt. Sta., State College, Miss. Jour. Anim. Sci., *3*, No. 1: 59–62. Feb., 1944.

Silage made from freshly cut sweet sorghum treated with about 10 pounds of urea per ton was compared with a lot untreated. The addition of urea speeded up the rate of fermentation, saved carotene and produced a silage with greater titratable acidity.

In a feeding trial with beef cows the urea-treated silage proved more palatable than the untreated. The cows that received the urea-treated silage maintained their weight while the untreated lot lost 47 pounds over a 78-day period. The urea-treated lot of cows were much better in appearance. C.F.H.

235. The Effect of Fat upon the Digestion of Nutrients by Dairy Cows. H. L. LUCAS AND J. K. LOOSLI, Cornell Univ., Ithaca, New York. Jour. Anim. Sci., *3*, No. 1: 3–11. Feb., 1944.

Two series of digestion trials using Holstein cows were conducted with rations of varying fat contents. In series one, there was no difference in the apparent digestibility of rations containing 1.6 and 2.6% ether extract.

In series two, using soybean products as the sole concentrates, rations containing 1.0 and 7.0% of ether extract were studied. The crude fiber and nitrogen-free extract of rations containing soybeans and solvent extracted soybean oil meal plus corn or soybean oil were less digestible than where the rations contained solvent-extracted soybean oil meal. The ether extract of rations containing soybean oil meal plus oil or fatty acids was more digestible than the ether extract of ration containing soybeans.

The ration containing soybeans caused a marked increase in fat percentage with practically no change in milk production. On the other hand, the rations containing either oil or fatty acids caused a pronounced decrease in milk flow, with little change in fat percentage. C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

236. Further Studies on the Comparative Value of Butterfat, Vegetable Oils, and Oleomargarines. R. K. BOUTWELL, R. P. GEYER, C. A. ELVEHJEM, AND E. B. HART, Dept. of Biochem., Univ. of Wis., Madison. Jour. Nutr., *26,* No. 6: 601–609. Dec., 1943.

When lactose was the sole carbohydrate in the diet, rats showed superior growth with butterfat or lard as compared to corn oil, coconut oil, cottonseed oil, soybean oil, peanut oil, olive oil and hydrogenated cottonseed oil. When a mixture of carbohydrates composed of sucrose, starch, dextrose, dextrin and lactose was in the diet, the average growth of the animals fed vegetable oils was equal to that of the animals fed butterfat or lard.

Properly fortified oleomargarine fats gave growth equal to butterfat over a period of 6 weeks with the above mixture of carbohydrates. Butterfat was superior to fortified oleomargarine when lactose was the sole carbohydrate in the diet. C.F.H.

237. Studies of the Comparative Nutritive Value of Fats. I. Growth Rate and Efficiency of Conversion of Various Diets to Tissue. H. J. DEUEL, JR., E. MOVITT, L. F. HALLMAN, AND F. MATTSON, Biochem. Dept., Univ. Southern Calif., Los Angeles. Jour. Nutr., *27,* No. 1: 107–121. Jan., 1944.

In three series of tests using male and female rats for periods of 6 to 12 weeks, the following fats were compared: a butterfat or whole butter, corn oil, cottonseed oil, or margarine fat or a whole margarine, olive oil, peanut oil or soybean oil. The basal diets used consisted of mineralized skimmed milk powder, with vitamins A, D and E. In one series whole butter and margarine were flavored by the addition to the fats of 4 parts per million of diacetyl.

In general the rats preferred a butter diet to one containing the vegetable fats. There was no difference, however, in the growth of weanling rats over a 12-week period when they were fed mineralized skimmilk powder, vitamin supplements and butter as compared with corn, cottonseed, olive, peanut or soybean oils or margarine. Also, the efficiencies of conversion of these various fats to body tissue were identical. The authors concluded that these results refute the idea that butterfat possesses certain saturated fatty acids not present in other fats, which are essential for growth. C.F.H.

ICE CREAM

238. **Frosted Foods and the Ice Cream Industry.** R. M. LAMBETH, Grand Rapids Cabinet Co. Ice Cream Field, *43,* No. 4: 34, 54, 56. 1944.

The author states that the first year following the war 150,000 ice cream cabinets will be built. He further predicts that there will be a buyer for all the cabinets produced for five years following the war. This prediction is made on the basis that a home freezer or storage cabinet is practical, economical and a convenient way to preserve foods.

It is stated that the past year has seen a large increase in demand for ice cream cabinets for the storage of frozen foods—many have been bought for the purpose of putting up "victory" garden products.

It is predicted that eventually "frozen foods will dominate the food field," and that the greater availability of cold storage cabinets will influence the future methods of distribution of ice cream.

It is claimed that there are 7,500 locker plants in the United States with 3,250,000 individual lockers which serve 3,000,000 families.

When restrictions on deliveries and equipment are removed the author expects there will be a marked increase in frozen food activity, including home delivery of such foods. He claims the dairy industry and especially the ice cream industry, has a distinct advantage in this field. W.C.C.

239. **The Melt Test.** B. I. MASUROVSKY, Res. Ed. Ice Cream Trade Jour., *40,* No. 4: 48. April, 1944.

A melt test, useful for determining characteristics of ice cream stabilizers is described and illustrated by photographs. In the test illustrated, 0.35% alginate was compared with 0.15% soluble soya lecithin. The latter produced a slower melting ice cream as indicated by less drainage and longer maintenance of shape. F.J.D.

240. **Injecting Pectinized Syrups, Ices or Sherbets into Ice Cream.** R. E. HAMILTON, Cleveland Ice Cream Co., Cleveland, Ohio. Ice Cream Trade Jour., *40,* No. 4: 30. April, 1944.

A mechanical injecting device is described for automatically incorporating various flavoring syrups, usually containing fruit or chocolate, into ice cream as it comes from a continuous freezer and moves toward the packaging point. A similar device is described for the injecting of previously frozen ices or sherbets into ice cream. The author uses a continuous freezer for pumping the material to be rippled, but notes that there is a pump on the market which should be capable of doing the job satisfactorily. F.J.D.

241. **Quality in Sherbets.** SAMUEL SABEL. Ice Cream Trade Jour., *40,* No. 4: 29. April, 1944.

The author issues a warning to ice cream manufacturers against a very common practice he has noted in all sections of the country during a recent business tour of the nation. The practice is that of concocting so-called sherbets from whatever materials are available when milk solids quota are exhausted merely to provide dealers with something to sell. In the author's opinion, this is a great mistake and one which not only reacts against future sherbet sales but will cause loss of confidence in the manufacturer which will carry over into the post-war period. He finds the more far-sighted manufacturers ceasing manufacture until the next period when it is not possible to manufacture to at least minimum standards. F.J.D.

242. Dried Whole Egg Powder. VIII. An Improved Fluorescence Method and Some Factors Affecting the Measurement. J. A. PEARCE, M. W. THISTLE, AND MARGARET REID, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *D. 21,* No. 11: 341. 1943.

This laboratory had previously reported a method for assessing egg powder quality by determining the fluorescence of a potassium chloride extract of the defatted powder. (See Jour. Dairy Sci. Abs., A220, 1943.) In order to save time and reagents the test has been modified as follows: 2.5 gm. of egg powder is defatted with three 25-ml. portions of chloroform; after drying at room temperature for about 1 hour, 1 gm. of the defatted powder is shaken for 30 minutes with 100 ml. of 10% sodium chloride solution, filtered, and the fluorescence of 15 ml. of the filtrate determined in the photofluorometer.

Increasing the temperature of the extraction raised the fluorescence values but pH changes between 4.6 and 8.9 caused no significant effect on results. O.R.I.

243. Dried Whole Egg Powder. IX. Effect of Drying Conditions on Quality. A. H. WOODCOCK AND MARGARET REID, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *D. 21,* No. 12: 389. 1943.

Liquid whole egg was spray-dried in a small laboratory drier at various rates of flow of liquid egg and at different inlet and exhaust air temperatures. Quality of the powder as assessed by chemical methods, palatability and baking tests, was progressively improved as the exhaust air temperatures were lowered. Inlet air temperatures above 225° F. had a deleterious effect. Lowering the drying temperature, however, had an adverse effect on the rate of production. O.R.I.

244. Dried Whole Egg Powder. X. The Effect of Added Substances on the Keeping Quality. JESSE A. PEARCE, A. H. WOODCOCK, AND N. E. GIBBONS, Natl. Res. Labs., Ottawa. Canad. Jour. Res., *F. 22,* No. 2: 34. 1944.

Dried whole egg powders, treated with a number of substances prior to drying, were stored at temperatures from 75° F. to 118° F. Deterioration in quality was assessed by fluorescence measurements, supported in some instances by palatability tests.

Fuorescence development in powders containing sodium chloride in combination with either citric or lactic acid was more rapid than in the control powder. The effect was less marked when any of these substances was used alone. The addition of 15% sucrose was more effective in inhibiting fluorescence development at 75° F. than at 99° F. but had no effect at 118° F. The addition of 0.2% sodium bicarbonate, an amount that did not affect the flavor of the powder, retarded deterioration as indicated by fluorescence and palatability tests. Other alkaline salts studied (sodium acetate, benzoate, citrate, salicylate and tartrate) had no effect. O.R.I.

245. **Ices and Sherbets.** R. J. RAMSEY, Ramsey Labs., Cleveland, Ohio. Ice Cream Field, *43,* No. 3: 8, 66. March, 1944.

The author gives an ice and a sherbet recipe and lists brief comments on some of the important problems related to the manufacture of ices and sherbets.

He states that 50 to 60% overrun is satisfactory for sherbets whereas 20 to 35% overrun should be maintained for ices.

He makes the following war-time recommendations regarding ices and sherbets:

1. Make at least 80% of the package ice cream as half ice cream and half sherbet or as sherbet ripple.

2. Sell as much bulk sherbet as possible.

3. Make at least 50% of the bulk gallonage as sherbet combinations—25 to 30% sherbet and 70 to 75% ice cream. W.C.C.

MILK

246. **Wild Onion and Garlic.** L. V. SHERWOOD. Univ. of Ill. Agr. Expt. Sta. Cir. 572. 8 pages. March, 1944.

Directions on how to recognize and control these noxious weeds. Cultural control is most effective; oil sprays can be used but the cost is high. J.G.A.

247. **Recent Developments in Milk Control.** C. K. JOHNS, Dept. of Agr., Ottawa. Canad. Jour. Pub. Health, *35,* No. 1: 33. 1944.

The efficient use of the laboratory may assist greatly the farm inspection staff and reduce travelling by the use of simplified tests for milk quality. These include the sediment test, dye reduction tests, and the direct microscope clump count. The resazurin test is a particularly good aid in detect-

ing milk from unhealthy udders. For the control of pasteurized milk the phosphatase and coliform tests are now coming into general use and are tending to supplant the agar plate method. O.R.I.

248. **Women in the Milk Industry.** T. J. HAMMELL, The Borden Co., New York, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 129, 1943.

Experience with women in plants was gained in the first World War. Union approval and cooperation was secured. When the same work was done the same pay was given as to men. An endeavor was made to secure women whose husbands were in the armed forces. More time was needed to break in the women workers as they were new to such work. The women are appreciative and do good work. A.C.D.

249. **Construction of Weighing Vats a Major Factor in Accurate Butterfat Sampling.** ELVIN R. ALBEE, Dept. of Health, Albany, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 125, 1943.

A study of milk weigh vats showed several that were not conductive to securing good mixing of the milk by dumping. A long narrow weigh vat gave samples that varied 0.4% in butterfat but the condition was helped by baffle plates. In another plant with rectangular two-compartment weigh cans the mixing was not good but the poorest mixing was in a weigh can with a strainer outlet near the bottom of the weigh can. In both these cases the mixing was good when the strainers were removed. The variations in test were both for and against the dealer. A.C.D.

250. **Milk Prospects for 1944.** FRED H. SEXAUER, Dairymen's League Coop. Assoc., New York, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 103, 1943.

This statement before the New York State Joint Legislative Committee deals with prices, feed supplies, numbers of cattle, and milk consumption with special reference to the New York area. All factors indicate a reduced milk supply in 1944 and the final prospects will be affected greatly by the program of the Federal Government. A.C.D.

251. **Cleaning and Sterilizing High-Temperature Short-Time Pasteurizers.** LEWIS SHERE, The Diversey Corp., Chicago, Ill. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 91, 1943.

It has been shown that the type of material to be removed from high-temperature pasteurizers is not the same as that found in vat pasteurizers. In vat pasteurizers the material is held together and to the equipment by casein; hence it can be removed by ordinary alkaline washing powders that

dissolve casein. In high-temperature pasteurizers the casein has combined with the mineral salts and cannot be so easily removed.

The best procedure for cleaning high-temperature pasteurizers is to cool the equipment by pumping through cold water until clear, then circulating an acid-base milkstone remover at 135°–150° F. for ½ to 1 hour, flushing out with warm water for 10 to 15 minutes, then circulating an alkaline cleansing solution at 135°–145° F. for ½ to 1 hour, and finally rinsing with cold water. Dismantle and brush with alkaline cleanser, if necessary. Just before using, rinse with warm water, assemble, and sterilize. Chlorine solution is preferred for sterilizing but hot water is excellent if temperature is properly maintained throughout the equipment. A.C.D.

252. **Trends in the Administration of the Supervision of Country Milk Supplies.** G. W. MOLYNEUX, Dept. of Health, White Plains, N. Y. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 7, 1943.

Recent developments have brought the public health inspection of milk into the economics of price regulation and the restriction of trade by regulation of the scope of producing areas. These two unwarranted activities together with duplications of inspections by cities could best be handled by placing all inspection of milk for both country and city under one central state milk inspection agency. A.C.D.

253. **Mutual Aid Between Pasteurizing Plants.** C. S. LEETE, N. Y. State Dept. Health, Albany. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 85, 1943.

As a war measure the New York State War Council as a central agency to prevent duplication and omissions set up an Office of Emergency Milk Supplies to arrange for the provision of milk to the public in case of a military disaster. The plan was based upon local and voluntary cooperation of milk distributors serving a given local area. The idea was that local cooperation would solve the emergency.

Although there have been no military disasters the plan has met emergencies satisfactorily. In one city a large milk plant burned about midnight. Milk was diverted to other plants, for one day other plants supplied customers of the burned plant, but thereafter the milk of the burned plant was processed by the other plants and delivered by the plant that had met with disaster. A.C.D.

254. **A Northeastern States Code for Milk for Pasteurization.** WALTER D. TIEDEMAN, N. Y. State Dept. of Health, Albany, N. Y. 17th Ann. Rpt. N. Y. Assoc. Milk Sanit., p. 63, 1943.

Much progress has been made in milk safety and quality by health officials and others. However, the tendency in recent years has been to

exceed the needs for a safe milk of good flavor, appearance, and keeping quality. There is need to unify regulations, and with this thought in mind health officials of eastern United States met and drew up "Northeast States Emergency Sanitation Standards for Raw Milk for Pasteurization." A copy of the regulations is presented. A.C.D.

255. Milk Plant Equipment in War-Time. O. K. BURROWS, Cherry-Burrell Corp., Chicago, Ill. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 51, 1943.

In 1942 manufacturers of dairy equipment went into war work and for the last two years replacements have been about 15% of normal. Not until 1944 has authorization been given to increase the manufacture of some dairy equipment. In addition to metal scarcity, there is the direct competition of war machines for parts such as nine motors of dairy size used on each Flying Fortress. There will be no immediate startling new equipment when the war is over. There may be some new metals and plastics and a broader experience gained in war work. A.C.D.

256. Dairy Cleansers and War Requirements. H. W. LEHMKUHL, Milk Plant Specialties, Rochester, N. Y. 17th Ann. Rpt. of N. Y. State Assoc. Milk Sanit., p. 37, 1943.

It is pointed out that cleansers have been greatly improved in the last few years, that the war-time shortage may become acute, requiring less concentrated solutions and more brushing to obtain clean equipment. A.C.D.

257. Labor in War-time for Dairy Farms and Milk Plants. A. D. GENTLE, Manpower Service, Albany, N. Y. 17th Ann. Rpt. of N. Y. State Assoc. Milk Sanit., p. 33, 1943.

This article discusses the manpower situation as it applies to dairy work. Some details of the Selective Service regulations are given. A.C.D.

258. The Effect of War-time Shortages upon Maintaining Sanitation on Dairy Farms and in Milk Plants. PAUL CORASH, N. Y. City Dept. of Health. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 23, 1943.

The war-time shortages in milk plants and on dairy farms are discussed under three headings: manpower, supplies and equipment. A.C.D.

259. War Time Increases Our Responsibilities. MILTON R. FISHER, D.V.M., Chief, Milk Control Dept. of Public Welfare, Health Div., St. Louis, Mo. Jour. Milk Technol., *6*, No. 6: 362. Nov.–Dec., 1943.

Some of the factors discussed which tend to increase inspection responsibilities are as follows:

Sudden increase in population in defense areas.

Increase in demand for milk.

Lack of new equipment to handle milk. Old equipment operated above capacity in some plants.

Greater need for sanitation.

Need to be careful about making concession that would jeopardize the dairy industry, the consumers as well as the milk sanitarians. L.H.B.

MISCELLANEOUS

260. Measurement of Detergency: Photometer for Determination of Films on Transparent Surfaces. Determination of Rate of Hard Water Film Formation in Washing of Glass Objects. JOHN L. WILSON AND ELWYN E. MENDENHALL, Economics Laboratory, Inc., St. Paul, Minn. Jour. Indus. and Engin. Chem., Analyt. Ed., *16*, No. 4: 251, 253. April, 1944.

The calcium and magnesium salts present in hard water react with many detergents to form insoluble compounds. In processes such as commercial dishwashing, some of the precipitate formed attaches itself to the objects being washed and builds up an unsightly film. A simple inexpensive photometer has been designed for the quantitative determination of this "hard water" film. Data are presented to show the reproducibility of results obtained by the suggested method and the ease and accuracy with which differences between detergents may be determined. B.H.W.

261. Analysis of Soap-Synthetic Detergent Mixtures in Bar Form. DONALD BERKOWITZ AND RUBIN BERNSTEIN, Detergent Section, Test Lab. U. S. Navy Yard, Philadelphia, Pa. Jour. Indus. and Engin. Chem., Analyt. Ed., *16*, No. 4: 239. April, 1944.

A procedure for the analysis of commercial soap-synthetic detergent mixtures is proposed which has given sufficiently accurate and reproducible results. The method is based on the separation of active ingredients from inorganic salts by means of ethyl alcohol, and the subsequent determination of soap, fatty matter, and sodium chloride in the alcohol-soluble portion. Synthetic detergent is calculated as the difference between total alcohol-soluble matter and the sum of soap, fatty matter and alcohol-soluble sodium chloride. B.H.W.

262. Stephen Moulton Babcock. H. H. SOMMER, Univ. of Wis., Madison, Wis. 17th Ann. Rpt. N. Y. State Assoc. Milk Sanit., p. 77, 1943.

This brief biography of Dr. Babcock is especially clear and thorough and ought to be read in its entirety. A.C.D.

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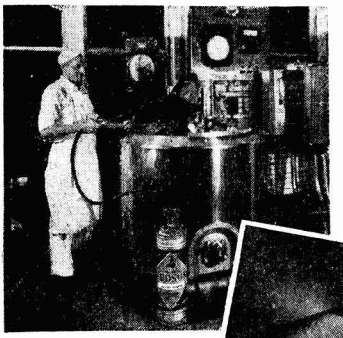

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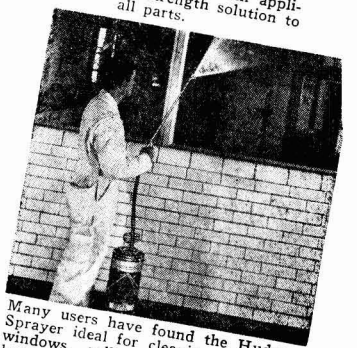

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