LACTOSE

A REVIEW

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HISTORICAL

Previous to the seventeenth century, milk was considered as composed of casein, butterfat, and serum. Fabritius Bartolettus, philosopher and physician of Mantua, wrote in 1619 (1), "In lacte sunt tres partes,—butyrum, serum, caseus." But in a later book (2), which he probably wrote in 1628, he mentioned a "manna seri" which he obtained by evaporation of milk serum. He spoke of it as "sal seri essentiale, seu nitrum," and described briefly its preparation and purification.

Ettmüller was the next of whom there is record to write of lactose. He described (3) in 1688 the evaporation of the whey and the purification of the crude lactose by recrystallization.

In Venice in 1694 Ludovico Testi (4) advertised lactose as an invention of his own under the name of "saccharum lactis" and advocated it enthusiastically as a remedy for gout and other diseases. The identity of the "saccharum lactis" of Testi with the "manna seri" of Bartolletus was pointed out by Fick (5) a few years later.

In India, lactose had been prepared previous to 1712, since Kaempfer (6) wrote of the "Brahmenes, qui etiam ex omnibus dulcibus, quin ex ipso lacte, saccharum eliciunt."

It was recommended by Stussius (7) in 1713 as an antiscorbutic, diuretic, and febrifuge, and its medicinal use was further discussed by Trostius (8) in 1739. It is interesting to note that most of the affections for which Dyvernois (9) recommended lactose are traceable to improper intestinal elimination and the accompanying autointoxication. He mentioned melancholy,

gout, inveterate itch (possibly urticaria), distemper, and hysterical passion.

In the first half of the eighteenth century, lactose was made on a commercial scale (10) from whey, and, in the last half of the same century, it became a recognized article of commerce (11).

In 1772, Lichtenstein (12) published the first monograph dealing with the chemical and physical properties of lactose.

SOURCES

Lactose occurs in the milk of all mammals with the possible exception of that of the whale. Scheibe (13) claimed that he could account for all the solids of whale's milk as protein, ash, and fat, and that the serum has no reducing action on cupric salts. Takata (14), however, has reported that the milk of Baleaneptera physalus, L., contains 1.8 per cent lactose.

Lactose has been reported from the fruit of Quercus racemosa (15) and from Achras sapota (16), but there is no corroboration for either statement.

It was believed as late as 1888 (17) that milks from different animals contained varying mixtures of different lactoses, but a careful comparison by Deniges (18) in 1893 of samples of lactose from the milks of a number of mammals showed that there is only one natural lactose—or, strictly speaking, one natural equilibrated mixture of stereoisomers—and that the abnormal polarization effects obtained from some milks were due to substances other than lactose. It has been claimed by Pappel and Richmond (19) that the milk of the Egyptian gamoose contains 5.56 per cent of a sugar that gives only *d*-glucose on hydrolysis. They named this sugar tewfikose.

The lactose content of the milk of various animals is given in table 1. Recent determinations have been selected when available. The single figures represent averages, the pairs of figures extreme values.

BIOSYNTHESIS

The problem of the mechanism of formation of lactose in the animal body has been a troublesome one, and even at the pres-

ent time the theory with the best support is not universally accepted. The problem offers three points for consideration: first, the place of fabrication; second, the agency of fabrication; and, third, the substance or substances from which lactose is formed.

It had been previously generally assumed that lactose is formed in the mammary gland, but the first real evidence was that of Bert (31), who removed the mammary glands of a goat and bred

TABLE 1

Percentage of lactose in milks from various sources

SOURCE	PERCENTAGE	AUTHORITY
Mare	4.32–7.56	Hildebrandt (20)
Mare	7.9	Folin, Denis, and Minot (21)
Elephant	7.27–7.39	Doremus (22)
Woman	5.49–8.35	Denis and Talbot (23)
Woman	7.06	Folin, Denis, and Minot (21)
Ass	6.86	Richmond (24)
Camel	5.78	Dragendorff (25)
Llama	5.60	Doyere (26)
Sheep	5.4	Folin, Denis, and Minot (21)
Buffalo	5.19	Tartler (27)
Goat	5.0	Folin, Denis, and Minot (21)
Cow	4.54	Folin, Denis, and Minot (21)
Hippopotamus	4.4	Cummings (28)
Indian buffalo	4.16	Dubois (29)
Hog	4.0	Folin, Denis, and Minot (21)
Cat	3.4	Folin, Denis, and Minot (21)
Guinea pig	3.0	Folin, Denis, and Minot (21)
Reindeer	2.21–2.80	Barthel and Bergmann (30)
Dog	2.6	Folin, Denis, and Minot (21)
Rabbit	1.8	Folin, Denis, and Minot (21)

the animal. After delivery, glucose appeared in the urine, which Bert took to indicate that the animal could not form lactose in the absence of the mammary gland and hence the gland must be the place of formation. This evidence has been corroborated many times.

Efforts to isolate from the mammary gland an enzyme capable of producing lactose from glucose and galactose have so far failed (32). However, a patent (33) has been issued for conversion of

glucose to lactose by means of a fluid obtained from the mammary gland.

Earlier evidence went to show that there exists in the functioning mammary gland a precursor of lactose analogous to, but not identical with, the glycogen of the liver (34), and one recent investigator (35) supports this idea. While specific evidence against this idea is rather limited, it apparently is not generally accepted.

Whether lactose is formed by a combination of glucose and galactose furnished as such from outside the body, or whether the process consists of a conversion of glucose to galactose, with subsequent or simultaneous combination with more glucose and dehydration, was in doubt up to the early part of the present century. An examination of the foods of herbivorous animals (36) indicated that these animals consumed enough galactose-containing compounds to account for the galactose portion of the lactose which they formed. However, since women and carnivorous animals consume insufficient galactose-yielding substances to account for the lactose which they produce, and since galactose is never found accompanying the glucose in the urine of recently delivered animals whose mammary glands have been previously removed (37), and since efforts to find any substance in the gland that could give galactose by hydrolysis were fruitless (38), the hypothesis of pre-formed galactose as a partial source of lactose has been rejected.

The theory that the mammary gland possesses the power of converting glucose to galactose was advanced three decades ago (39) and has had strong support more recently (40). The considerable amount of work carried out in recent years by Porcher (37) (40) (41) and others (42) brings out the following facts:

1. Removal of the mammary glands before or during pregnancy causes glucose to appear in the urine immediately after delivery; lactose or galactose do not appear.

2. Women who excrete glucose in the urine for a short time before delivery, but who are not true diabetics, excrete lactose in place of the glucose immediately after delivery.

3. Removal of the mammary glands during lactation causes temporary hyperglycemia and glucosuria.

This evidence indicates that lactose is formed in the mammary gland, that its source is exclusively glucose, and that the glucose is supplied from the liver by way of the blood stream. This implies a condensation of pairs of glucose molecules accompanied by a simultaneous internal rearrangement of one member of the glucose pair to a galactose structure.

DISTINGUISHING TESTS

Oxidation of lactose by dilute nitric acid (43) serves to distinguish it from all other sugars except galactose and such as contain a galactose residue. Mucic acid is formed which may be identified by its slight solubility or its titration value (44).

Lactose may be distinguished from glucose and galactose by the use of Barfoed's copper acetate solution (45) or an ammoniacal copper sulfate solution (46), both of which are reduced by the monosaccharides but not by the lactose.

The phenylosazone of lactose cannot be easily distinguished from those of glucose and galactose by its melting point, but it may be identified microscopically (47). Another method (48) of identification is to determine the rotation of 0.2 gram of the osazone in 4 cc. pyridine and 6 cc. alcohol. It should be zero.

Of the various color reactions of the sugars with phenols (49), the yellow-red given by lactose with resorcin distinguishes it from sucrose, which gives a bright red. Phloroglucin gives a red-brown with lactose, a yellow-red with sucrose.

Lactose solution heated with lead acetate (50) becomes yellowish after several minutes. If ammonia solution is added drop by drop, a precipitate forms and redissolves for a time. Then appears a brick-red coloration, next a cloudiness, and finally a cherry-red precipitate with a colorless liquid above it.

A drop of pure diphenylhydrazine heated with a few mg. of lactose and 2 to 3 drops of glacial acetic acid (51) gives a color change from violet through yellow-red and brown-red to dark green. If finally a few cubic-centimeters of diluted alcohol are

added, a characteristic green solution is obtained. This reaction is said to take place even in the presence of other sugars.

o-Tolylhydrazine gives no hydrazone with lactose itself (52a) but does with its hydrolytic products. It is a specific reagent for a galactose configuration with a functioning CO group.

Mycological methods have been advocated for distinguishing sugars, particularly those occurring in urine (52b).

DETERMINATION[1]

The uncertainties and difficulties in the determination of lactose in particular and reducing sugars in general are indicated by the large amount of work that has been done in this field. Only in the earliest methods was the lactose itself weighed as such, and then only in impure condition. Optical properties and reducing power are the bases of practically all modern methods and all such methods are affected by the presence of protein and other sugars. The lack of stoichiometric relationships in most reducing methods is a further factor that requires careful standardization of manipulations.

Of the substances recommended for removal of protein from milk before lactose determination, there may be mentioned basic lead acetate (53), acetic acid (54), copper acetate (55) (for which is claimed the advantage of removing the dextrin-like substance that often interferes with the polarimetric determination) mercuric nitrate or iodide (56), metaphosphoric acid (57), picric acid (58), asaprol (59), aluminium hydroxide (60), zinc ferrocyanide (61), ammonium sulfate (62), and colloidal iron (63). The Methods of the Association of Official Agricultural Chemists, 1920, recommends mercury or copper compounds.

Either the polarimetric or cuprimetric methods, or a combination of both, may be used for mixtures of lactose with sucrose, invert sugar, or maltose. The determinations may be applied before and after partial or complete inversion, or before and after

[1] Since this article was written, there has appeared an extensive descriptive summary of methods applicable to lactose determination together with an experimental comparison of the principal methods; Bleyer and Steinhauser, Milchwirtschaftliche Forschungen, **1**, 131–199, (1924).

fermentation (64). Sucrose may be inverted by the use of citric acid without affecting lactose (65). Brewer's yeast may be used to destroy all common sugars but lactose (66). Bacterium paratyphosi B. will destroy glucose and leave lactose unchanged (67). Lactobacillus bulgaricus will destroy lactose without affecting sucrose (68). Saccharomyces anomolus Hansen will completely remove maltose (69). The proper choice to make among these schemes of separation will be clear from the demands of any particular problem.

Considering now the methods of determination themselves, if we disregard the earlier direct methods such as that of Haidlen (70), we come chronologically to the work of Poggiale (71) (53) and of Fehling (72). Poggiale developed a polarimetric scheme which did not have much success till Ritthausen in 1878 (55) and Wiley in 1884 (56) applied copper and mercury salts respectively to the task of complete removal of protein from milk. Another difficulty with the polarimetric scheme is due to the error arising from the change in volume caused by the removal of precipitated protein. This has been solved in several ways. Wiley and Ewell (73) recommend polarizing solutions of two different dilutions and dividing the product of the two readings by their difference. Others have derived formulae for the conversion of observed to true values (74), and still others have calculated the excess volume of sample to allow in order to neutralize the error (75). For working directions for this method, those of the Association of Official Agricultural Chemists (76) will be found convenient.

Poggiale also developed the volumetric cuprimetric method (71), employing a single solution containing copper sulfate, potassium tartrate, and potassium hydroxide (77) (78). The chief variations in this reagent have been in the substitution of ammonium hydroxide (79) and of alkali carbonate (80) for potassium hydroxide, and of citrate (80) for tartrate. Benedict's method is very popular for reducing sugars and has been successfully applied to the determination of lactose in milk (81). Folin and Denis (82) have employed a volumetric copper solution containing phosphates for the determination of lactose in small samples of

milk and urine. The iodimetric determination of the excess of unreduced copper is also a somewhat popular method (59) (83).

In the gravimetric field, Fehling (72) developed the idea of keeping the copper sulfate and the alkaline tartrate solutions separate till just before using, but was unable to obtain a constant gravimetric ratio between lactose and copper, though he worked at the problem for twelve years (84). Soxhlet (85) accepted the variation in ratio and found it a gradual variation depending upon a number of factors. He drew up the first of many tables for the conversion of weight of copper to weight of lactose. Much work on gravimetric methods has been done since that time, largely on standardization of conditions and on the forms in which the copper may be weighed. Among these may be mentioned the investigations of Walker (86), Peters (83a), Quisumbing and Thomas (87), and Elsdon (88).

Several colorimetric methods for lactose determination have been devised. Among the colorimetric reagents are sodium hydroxide (89), ammonium hydroxide (90), and picric acid (91).

The Wollny refractometric method (92) is rapid and accurate for fresh normal or watered milk, but is not reliable for milks from sources other than cows (93). An immersion refractometer graduated to read directly in terms of lactose (94) is very convenient for this determination.

The oxidation of lactose to mucic acid by nitric acid may be used as the basis of a method of determination (95), but, since conditions of oxidation and crystallization have a considerable influence on the amount of mucic acid obtained and since only a portion of the mucic acid theoretically possible is ever gotten, it is rather a rough and unreliable method for quantitative work (96).

Oxidation of lactose by potassium permanganate (97) has been employed quantitatively, as has a method based on the oxidation of lactose to lactobionic acid by hypoiodite (98).

HYDROLYSIS AND UTILIZATION

Lactose may be hydrolyzed by the lactase of the intestines of mammals—particularly young mammals—(99), by the lactase

present in almonds (100) and in certain yeasts such as Saccharomyces tyrocola and the Saccharomyces kephir of kephir grains (101), and by dilute acids (102).

For sometime it has been generally agreed that lactose is not directly assimilated by the animal organism, but is hydrolyzed by a lactase to hexoses, which are directly utilized (103). Dastre was awarded the Prix Montyon in 1883 (99) for his researches on this problem. His conclusions were that lactose is utilized through conversion to hexoses, that this conversion occurs in the small intestine, that the agent is the intestinal fluid, that galactose and glucose are the hexoses formed, that these are utilized in the nutritional exchange, and that they may under certain conditions recombine to form lactose. However, Bourquelot and Troisier (103d) fed lactose to a diabetic and recovered in the urine two mols of glucose for each mol of lactose fed and found no galactose. This can be accounted for only by assuming a secondary conversion of galactose to glucose in the digestive tract, since animal lactases in the laboratory convert lactose into equal quantities of glucose and galactose (104). It should be remembered in this connection that in the formation of lactose the reverse change—glucose to galactose—is involved.

Searches for the enzyme hydrolyzing lactose have failed to prove its presence in the pancreatic fluid, the liver, or the stomach (105). It is found plentifully in the small intestine of young mammals, probably being formed at or in the intestinal wall (104a) (106). It diminishes in amount or even disappears with age. It is secreted by birds that have been fed on lactose, though it is ordinarily entirely absent from their digestive tract. Evidence is conflicting as to the point in the digestive tract of birds where the lactase is formed (106a) (107), but its source is probably the crop. It is interesting to note that the intestinal fluid of certain snails is particularly rich in lactase (108).

The fact that beer yeast does not ferment lactose was noted over one hundred years ago (109). That this was due to the inability of the yeast to invert lactose, and that the action of molds was analogous, was not brought out till the time of Berthelot (110) and Fitz (111). Influenced probably by the fact that

alcoholic drinks *were* produced from milk by wild yeasts and by the idea that therefore some yeasts *ought* to ferment lactose, many experimenters tried, without success, to isolate pure yeasts that would ferment lactose. Duclaux in 1887 (112) and Adametz in the following year (113) each reported the isolation of such a yeast. Duclaux's yeast is identical with Saccharomyces tyrocola, and Adametz's Saccharomyces lactis is the same as Saccharomyces kefir (101). A specific lactase is present in both these yeasts, but it is absent from beer yeast. Lactase exists in almonds together with emulsin (114). It occurs also in many seeds, particularly those of the Rosaceae and Cruciferae species (115). The optimum temperature for lactase activity is 37–38°. It is sensitive to phenols, acids, and alkalies.

The acid hydrolysis of lactose will be mentioned again later, but it should be noted here that the weak organic acids do not hydrolyze lactose so readily as they do sucrose (65). For the complete inversion of the lactose in 100 cc. of a 5 per cent solution, Saillard (116) recommends that 10 cc. of 36 per cent hydrochlorid acid be used and the solution be held at 90° for ninety minutes.

BACTERIOLOGICAL

The chief interest of chemists in the bacterial fermentations of lactose lies, not in the species of bacteria that attack this sugar, but rather in the chemical changes brought about by various organisms. Though traces of very many compounds may be found in lactose solutions that have been subjected to bacterial action, the substances formed in any considerable amount are comparatively few.

The lactic acid fermentation (117) is probably the most important and is caused by a number of organisms, particularly those occurring in milk, the most common of which is Streptococcus lactis. This organism is the cause of the normal souring of milk, it is used to hasten the ripening of cream, and contributes to the flavor of many dairy products. Bacterium caucasicus is a lactic organism of some chemical interest (118) because it accompanies the yeast of kefir grains. Lactobacillus acidophilus and

Lactobacillus bulgaricus are lactic acid producers which are used to combat putrefactive organisms in the intestines. These last two will be mentioned later in connection with the use of lactose in the diet. The lactic acid of fermentation may be dextro, levo, or inactive; and it may or may not be accompanied by appreciable amounts of by-products, these differences depending on the type of bacteria concerned (119).

Among the other products of bacterial action on lactose may be mentioned ethyl alcohol (120), viscous gums (121), methane, acetone, acetic acid (122), carbon dioxide, hydrogen (123), butyric acid (121b), (123) (124), acetaldehyde (124a), butyl alcohol (121b) (124b) (125), succinic acid (126), and propionic acid (127). Of these, butyl alcohol, lactic, propionic, butyric, and acetic acids are the only ones formed in any quantity. The commercial utilization of lactose for the manufacture of these substances is impracticable as long as cheaper sugars are available.

The use of lactose by the bacteriologist is largely confined to work with the colon-typhoid-dysentery group of organisms. The bacteria fermenting lactose have been discussed extensively by Levine (128) from the bacteriological standpoint. He defines the colon group as including non-sporing, Gram-negative bacilli which ferment lactose with the production of acid and gas and which are capable of growing aerobically. The typhoid and dysentery types do not ferment lactose, a fact which is made use of in preparing differential media for the isolation of these pathogenic organisms from the members of the colon group which invariably accompany them. The presence of members of the colon group in water supplies is generally assumed to indicate sewage pollution, soil contamination, or proximity of pollution. The members of this group most frequently found in water are B. coli communis and B. lactis aerogenes. B. coli communior and B. acidi lactici are also important members of the group. B. lactis aerogenes and B. acidi lactici are frequently present in milk and contribute to its souring. Spore-forming lactose fermenters are sometimes met with in water samples and of course interfere with the presumptive test for the colon group.

OXIDATION AND HYDROGENATION

A great many different substances may be obtained from lactose by oxidation. The products formed are determined by the choice of oxidizing agent, concentration of oxidizing agent, and the physical conditions of the oxidation.

The auto-oxidation of lactose in the presence of dilute acid (129) results in levulinic acid (γ-ketovaleric), $CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot COOH$, and formic acid. This reaction may be accounted for by assuming that hydrolysis to the hexoses takes place first, followed by an interchange of oxygen atoms between various groups in the hexose molecules and a splitting out of formic acid and water.

Iodine and lactose heated together in a sealed tube in a steam bath (130) yield formaldehyde, formic acid, and a yellow humic substance which has no reducing power and contains no iodine. Bromine (131) oxidizes the terminal aldehyde group of lactose to carboxyl, thereby forming lactobionic acid, $C_{12}H_{22}O_{12}$. The hydrobromic acid formed simultaneously tends to hydrolyze the lactobionic acid to galactose and gluconic acid, and continued action of bromine gives as final products galactonic and gluconic acids (132). Oxidation of the calcium salt of lactobionic acid (133) by hydrogen peroxide removes the terminal carboxy group and oxidizes the adjacent alcohol group to an aldehyde group, thus producing galactoarabinose, $C_{11}H_{20}O_{10}$.

Lactose is apparently not attacked by ozone (134) or hydrogen peroxide (135) in neutral or acid solution, but is oxidized in alkaline solution. Even air is able to oxidize sugars in alkaline solution (136), hydroxy acids and formic acid being the products. Considerable work has been done by Kiliani and his associates (137) and by Nef (136c) (138) on the various saccharinic acids and their lactone anhydrides, the saccharins. Kiliani's work has been mainly on the products of air oxidation of lactose in the presence of milk of lime. He has identified among these products, α-methylol-α-γ-δ-trihydroxypentoic acid, α-γ-δ-ϵ-tetrahydroxyhexoic acid, and α-ethyl-(ω-ol) α-β-γ-trihydroxybutyric acid. By oxidizing with nitric acid unidentified products of the

reaction, he obtained *d*-tartaric acid, a normal trihydroxyadipic acid, and a tribasic acid, $C_6H_8O_8$.

Solutions of cupric salts react with lactose to give a variety of products; among them, carbon dioxide, formic, lactic, and glycollic acids (139).

The oxidation of lactose by nitric acid to give mucic acid was an early known reaction (140). It has been used as a rough quantitative method of determination of lactose (95), and is a convenient qualitative test for galactose and galactose-containing compounds. The chief products of this oxidation (43) (141) when dilute—25 to 35 per cent—nitric acid is used are the isomeric tetrahydroxyadipic acids, saccharic and mucic, the former derived from the glucose portion of the molecule, the latter from the galactose portion. Tartaric and racemic acids are formed to a slight extent, the tartaric being formed probably by the further oxidation of the saccharic acid, the racemic by the oxidation of the mucic acid. Oxalic and carbonic acids are produced by nitric acid of higher concentration at the expense of the mucic and saccharic acids.

Potassium permanganate in hot acid or alkaline solution oxidizes lactose to carbon dioxide and water (142). However, if the permanganate be of low concentration in the acid solution and be added slowly, considerable quantities of formic acid may be obtained (143). Lactose oxidized with chromic acid is reported to give about 10 per cent of its weight of furfural (144).

Treatment of lactose with sodium amalgam has a number of effects (145). The hydrolysis to hexoses apparently is complete. Hydrogenation takes place to a considerable extent, converting the hexoses into dulcitol and mannitol (sorbitol?). Intramolecular rearrangement, reduction, and further splitting take place to a slight extent, yielding small amounts of sodium lactate, isopropyl alcohol, ethyl alcohol, and hexyl alcohol. The treatment of a water solution of lactose with hydrogen in the presence of nickel (146) gives lactositol, dulcitol, and sorbitol.

CHEMICAL EFFECTS OF HEAT

Lactose hydrate may be heated to 110° without change, but between 110° and 130° it loses all its water of crystallization.

The anhydrous lactose becomes yellow in color at 150 to 165° with no perceptible change in weight (147). At 175° it becomes brown, emitting a characteristic odor and losing about 13 per cent of its original weight. If the heating is not carried above this last temperature, a reaction mass is obtained containing some anhydrous lactose, a substance insoluble in water, and lactocaramel which is water-soluble. For isolation of the caramel, the mass is powdered, warmed with alcohol, and the lactose filtered off. The filtrate is evaporated to a syrup, diluted with water, and again filtered. The filtrate is evaporated to dryness and dried at 100°. Analysis indicates lactocaramel to have the empyrical formula $C_{12}H_{20}O_{10}$. It reduces chromic acid rapidly. It gives no precipitate with barium hydroxide solution, which distinguishes it from sucrose caramel. With ammoniacal lead acetate solution, lactocaramel gives a coffee-colored precipitate insoluble in water or alcohol, but soluble in acid. Apparently the same substance has been obtained recently (148) by dehydration of lactose at 185° for ten to twelve hours under 4 to 6 mm. pressure. The name lactosan has been applied to this compound; constitutionally it is probably 5-galactosyl-glucosan. It polymerizes somewhat during formation and readily in the presence of zinc chloride at 105°.

Pyrocatechin has been identified as one of the products of heating a lactose solution to 280° in a sealed tube (149). If the heating be carried out in a retort with sodium hydroxide solution, a considerable quantity of lactic acid and small amounts of formic acid and pyrocatechin are formed. Fusion of lactose with potassium hydroxide gives small amounts of succinic acid (150).

DERIVATIVES

The phenylhydrazones and phenylosazones of sugars have been of considerable use in distinguishing the members of the sugar group from one another ever since their discovery by Emil Fischer. Lactose phenylhydrazone (151) may be prepared by dissolving one part lactose in one part hot water and, after cooling, adding one-half part phenylhydrazine. After two days standing, this mixture is added to twice its volume of absolute

alcohol. Ether is then added and causes the separation of a yellow syrup. This may be obtained as a colorless solid by redissolving in absolute alcohol and reprecipitating with ether. It is levorotatory. As a class the phenylhydrazones are slightly soluble in water and alcohol. Following is a list giving the properties of a few lactose phenylhydrazones with substituent groups (152):

Lactose amylphenylhydrazone, brown, M.P., 123°, $(\alpha)_D = -8.6°$ in alcohol.
Lactose allylphenylhydrazone, yellow, M.P., 132°, $(\alpha)_D = -14.6°$ in alcohol.
Lactose benzylphenylhydrazone, yellow, M.P., 128°, $(\alpha)_D = -25.7°$ in alcohol.
Lactose β-naphthylhydrazone, brown M.P., 203°, $(\alpha)_D = 0$ in alcohol, + 7° in glacial acetic acid.

Lactose phenylosazone is made (153) by heating together 1 part lactose, $1\frac{1}{2}$ parts phenylhydrazine hydrochloride, 2 parts sodium acetate, and 30 parts water. On cooling, the osazone separates out as fine yellow needles, melting at 200°, soluble in 80 to 90 parts boiling water, somewhat more soluble in hot alcohol, very soluble in hot glacial acetic acid, insoluble in benzene, ether, or chloroform. Dehydration of the osazone by means of 20 per cent sulfuric acid yields an anhydride which is insoluble in water, ether, or benzene. From hot absolute alcohol it may be crystallized as yellow needles, M.P., 223° to 224° (uncorr.). Phenyllactosazone treated with 5 parts of cold fuming hydrochloric acid (154) decomposes into phenylhydrazine and lactosone $C_{12}H_{20}O_{11}$, in which the secondary alcohol group adjacent to the aldehydic group of lactose has become a carbonyl group. Phenyllactosazone dissolved in a mixture of pyridine and alcohol shows no optical rotation (48). It may be mentioned here that an isolactose has been synthesized from glucose and galactose by the action of kefir lactase (155), the phenylosazone of which melted at 190° to 193°.

Literature records tri-, tetra-, penta-, hexa-, and octonitrates as resulting from the action of nitration mixtures on lactose (156). The octonitrate is probably the best authenticated (157). It melts at 145–146°. $(\alpha)_D^{20°} = + 74.2°$ in 2 per cent solution in methyl alcohol.

Lactose octoacetate, or octoacetyllactose, was first prepared

in 1869 (158) by boiling lactose with acetic anhydride. Herzfeld (159) prepared it later in crystal form by acetylation with acetic anhydride and anhydrous sodium acetate. Proof of the existence of two isomers and the establishment of their properties was only recently accomplished by Hudson and Johnson (160). They acetylated by the second method and obtained octoacetyl-β-lactose which crystallized as microscopic crystals from alcohol and melted to a viscous liquid at 90°. $(\alpha)_D^{20}$ D = − 4.4° in 10.6 per cent solution in chloroform, −23.5° in 10.5 per cent solution in benzene, and 0° in acetic acid. The β compound dissolved in glacial acetic acid containing zinc chloride changes slowly to the α form, which has a higher solubility in ether and alcohol. It crystallizes as fine felted needles and melts at 152°. $(\alpha)_D^{20}$ = + 54° in 10.6 per cent solution in chloroform, +28.6° in 10.6 per cent solution in benzene, and +59.9° in 10 per cent solution in glacial acetic acid. The equilibrium mixture in glacial acetic acid contains about 86 per cent α and 14 per cent β. Both octoacetates, on treatment with hydrobromic acid, give the same heptaacetylbromlactose. This brom compound gives only the β-octoacetate when treated with silver acetate (161).

Heptaacetylbromlactose may be prepared from lactose and acetyl bromide (162), but the two-stage method is more convenient (163). Octoacetyllactose dissolved in acetic anhydride is mixed with a saturated solution of dry hydrobromic acid in glacial acetic acid. After one and one-half hours the mixture is poured into ice water. The precipitate is dissolved in chloroform, the chloroform solution washed with water and dried, and the substance reprecipitated by addition of petroleum ether. By crystallization from warm alcohol, it is obtained as prisms melting at 143° to 144° (corr.). In chloroform solution, $(\alpha)_D^{22}$ = + 104.9°; in acetylene tetrachloride, $(\alpha)_D^{22}$ = + 105.16°. Heptaacetylbromlactose is insoluble in petroleum ether, slightly soluble in water, fairly soluble in chloroform, readily soluble in alcohol, benzene, ether, toluol, acetone, and ethyl acetate. It reduces Fehling solution after short heating. Treatment with silver carbonate and methyl alcohol gives heptaacetylmethyllactoside, M.P., 66° to 67°. $(\alpha)_D^{19}$ = − 5.91° in chloroform

solution. Treatment of heptaacetylbromlactose in chloroform solution with silver carbonate and menthol (164) gives heptaacetyl-β-menthollactoside, M.P., 125° to 130°., readily soluble in the usual solvents with the exception of water, petroleum ether, and ligroin, $(\alpha)_D^{19} = -29.65°$ in acetylene tetrachloride solution. Saponification of this compound gives the β-menthollactoside, which crystallizes with 4 molecules of water, M.P., 110°, $(\alpha)_D^{16} = -38.04°$ in acetylene tetrachloride solution. The Fischers claim to obtain a tetradekaacetyltetrasaccharide from heptaacetylbromlactose and silver carbonate, but Hudson and Sayre (165) report a heptaacetyllactose, M.P. 83° (corr.). A thiophenollactoside is obtained by treating heptaacetylbromlactose with sodium thiophenylate in ether solution and saponifying off the acetyl groups (166). In water solution $(\alpha)_D^{20} = -40.0°$. Its melting point is 221° (corr.). It dissolves readily in cold water and its solution has a bitter taste. It is very slightly soluble in ether, ethyl acetate, or chloroform. Hydrolysis gives galactose and thiophenol glucoside.

Reduction of heptaacetylbromlactose by zinc and acetic acid (167) involves two secondary alcohol groups and gives hexaacetyl lactal, $C_{24}H_{32}O_{15}$, an unsaturated compound. Saponification of hexaacetyl lactal by barium hydroxide or methyl alcoholic ammonia (168) gives lactal, $C_{12}H_{20}O_9$. It may be obtained as the monohydrate or the anhydrous substance. Lactal is faintly sweet, dissolves readily in water, slightly in hot alcohol or acetone, and not at all in ether or chloroform. It restores the color to fuchsin-sulfurous acid solution and decolorizes bromine water. It is hydrolyzed by lactase to galactose and glucal. Boiling hexaacetyl lactal with water gives a pentaacetyl pseudolactal, which on reacetylation gives hexaacetyl pseudolactal. Saponification of pentaacetyl pseudolactal gives isolactal. Acetylation of isolactal gives hexaacetyl isolactal. The three hexaacetyl compounds differ widely in melting points. Hydrogenation converts hexaacetyl lactal into hexaacetyl hydrolactal. This on saponification becomes hydrolactal, $C_{12}H_{22}O_9$. This compound may also be obtained directly from lactal by hydrogenation. It is slightly sweet, dissolves in twice its weight of water, is

somewhat soluble in hot methyl alcohol, but only very slightly soluble in the other common solvents. It does not show the reactions of an unsaturated compound nor does it reduce Fehling solution. It may be hydrolyzed to galactose and hydroglucal.

Heptaacetylchlorlactose may be prepared by treating lactose with acetic anhydride and dry hydrochloric acid in one operation (169), or by treating octoacetyllactose with dry hydrochloric acid (170). It exists in two modifications, one soluble in ligroin and melting at 57° to 59°, $(\alpha)_D^{20} = +76.2°$, the other insoluble in ligroin and melting at 118° to 120°, $(\alpha)_D^{20} = +73.5°$. The first is probably the α compound and the second the β, since the second gives the β-octoacetyllactose by treatment with silver acetate. The higher melting modification treated with silver carbonate and methyl alcohol (161) gives heptaacetylmethyllactoside, M.P., 65° to 66°, $(\alpha)_D^{19} = +6.35°$ in chloroform. Gentle saponification removes the acetyl groups leaving methyllactoside, M.P., 170° to 171°.

Preparation of the β-acetyliodolactose has been reported by Mills (171). It is claimed that butyryllactose has been prepared by heating lactose with butyric acid (172).

Heptabenzoyllactose may be prepared by treating lactose with benzoyl chloride in the presence of alkali (173). It crystallizes in small rods which melt at 200°. Apparently some penta-, hexa-, and octobenzoyl compounds are also obtained. Hexabenzoyllactose has been reported as melting at 130° to 136° (174).

Aniline and lactose combine with the elimination of water to form a monolactose monoanilide (175). This compound is readily soluble in water, acids, alkalies, dilute alcohol, alcoholic aniline, and alcoholic ammonia. It is slightly soluble in absolute alcohol and insoluble in ether, carbon disulfide, chloroform, and benzene. It reduces alkaline copper solutions and is decomposed in aqueous solution by bromine. $(\alpha)_D = 14.19°$ in water solution. A toluide of lactose has also been prepared.

Lactose is insoluble in ethyl alcoholic ammonia solution, but dissolves in methyl alcoholic ammonia solution, from which after about a fortnight lactose ammonia (176), $C_{12}H_{22}O_{11} \cdot NH_3$, separates as small needle crystals. $(\alpha)_D = -39.5°$. It slowly decomposes into lactose and ammonia in the presence of sulfuric acid.

That a molecular compound of pyridine and lactose exists in solution is indicated by the solubility of lactose in various pyridine-water mixtures (177).

Lactose combines with aminoguanidine salts (178) to form dextrorotatory compounds, – $(C_{12}H_{22}O_{10}\cdot CN_4H_4)_2\cdot H_2SO_4\cdot 7H_2O$, $C_{12}H_{22}O_{10}\cdot CN_4H_4\cdot HNO_3$. The nitrate forms microscopic needles melting with decomposition at about 200°.

Lactose ureide (179), $C_{12}H_{21}O_{10}\cdot NH\cdot CONH_2\cdot H_2O$, may be prepared from lactose and urea in sulfuric acid solution. It crystallizes in monoclinic needles or plates from 50 per cent alcoholic. $(\alpha)_D = +2.1°$. At 230° it turns brown and at 240° it decomposes. It may be acetylated.

Lactose semicarbazone (180), $C_{12}H_{22}O_{10}{:}N\cdot NH\cdot CO\cdot NH_2\cdot 2H_2O$, loses one molecule of water at 115°, the second above 120°, and melts at 185°. Initial $(\alpha)_D = +10.6°$ in water solution, becoming +11.25° after 24 hours. It is soluble in water to the extent of 19.8 per cent at 20.5°.

The octophenylurethane of lactose (181), $C_{12}H_{14}O_{11}(CONHC_6H_5)_8$, melts at 275–280° and does not reduce Fehling solution.

Ethyl mercaptan reacts with lactose (182), but the lactose mercaptan has not been isolated. Apparently it immediately hydrolyzes to the hexose compound. Hydrogen sulfide does not react with lactose appreciably (183).

By passing carbon dioxide into mixtures of calcium hydroxide and lactose, products containing equimolecular amounts of calcium carbonate and lactose have been obtained (184).

Several patents (185) claim that lactose and formaldehyde form definite compounds, but more recent and extended experiments (186) have indicated that only mixtures of variable composition are obtainable.

By means of the cyanhydrin reaction, lactose may be converted to lactose carboxylic acid (187), $C_{12}H_{23}O_{11}\cdot COOH$. This acid is readily soluble in water, slightly soluble in alcohol, and insoluble in ether. It does not reduce Fehling solution, it forms an insoluble basic lead salt, and on reduction yields a sugar, $C_{13}H_{24}O_{12}$. Hydrolysis converts lactose carboxylic acid into galactose and glucoheptonic acid.

Compounds of lactose with metals have been reported (188), but their properties have not been well defined.

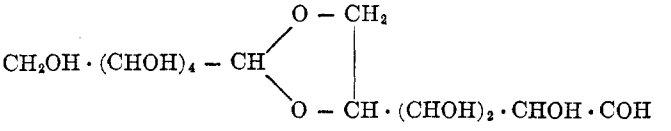
STRUCTURE

The discussion of the structure of lactose will be limited in this paper to the identity of the hexose sugars involved and their mode of linkage.

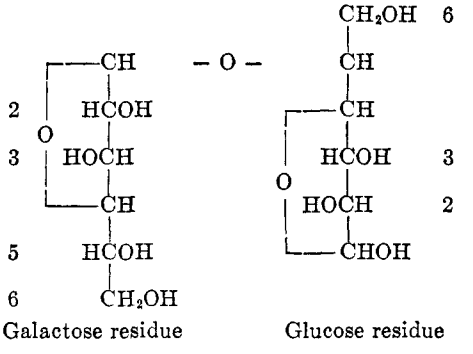
The earliest record of the hydrolysis of lactose is by Vogel (102) in 1812. He obtained from lactose and acid a sweet crystalline substance which he supposed was glucose. Erdmann in 1855 (189) showed that lactose on hydrolysis gave a sugar whose specific rotation was not equal to that of glucose. Liebig in 1856 (190) noted the aldehydic reducing action of lactose. Pasteur in the same year (191) found that the sugar which he isolated from the hydrolytic products of lactose gave on oxidation twice as much mucic acid as the same weight of lactose and consequently could not be glucose, but must be a new sugar. Fudakowski in 1866 (192) established the fact that *two* sugars were formed by the hydrolysis of lactose. He found that these sugars differed in crystal form and specific rotation, and that one gave mucic acid on oxidation with nitric acid, but the other did not. He later established differences between the melting points and reducing powers of the two hexoses and applied the names galactose and lacto-glucose (193). The ratio between the elementary components of lactose was first correctly established by Stadeler and Krause in 1854 (194).

In 1888, Emil Fischer (154) converted phenyllactosazone to lactosone by means of fuming hydrochloric acid. Hydrolysis of lactosone gave glucosone and galactose. At about the same time, he and a co-worker (131) hydrolyzed lactobionic acid to gluconic acid and galactose. Fischer reasoned that since lactose forms a monobasic acid on mild oxidation, it must contain only one of the aldehyde groups of its constituent hexoses. It is this aldehyde group that becomes the carboxyl group of lactobionic acid and on hydrolysis, the carboxyl group of gluconic acid. Therefore the aldehyde group of lactose belongs to the glucose portion of the molecule and, on the other hand, the aldehyde group of galactose does not exist as such in lactose, but must

be the point of union of the galactose residue to the glucose residue. Since the aldehyde group and the adjacent secondary alcohol group of lactose must be the groups concerned in lactosazone formation and consequently must become the $-CO \cdot COH$ grouping of Fischer's lactosone, the same conclusion as that above may be drawn from the hydrolysis of lactosone to glucosone and galactose. This conclusion has been further verified by the hydrolysis of various other lactose derivatives to galactose and the corresponding glucose derivatives (108) (133) (167b) (187b), and by work of van der Haar (52), who found that *o*-tolylhydrazine, which is a specific reagent for a galactose configuration with a functioning carbonyl group, gave no hydrazone with lactose. Fischer offered in his paper mentioned above the following linkage for lactose:



Haworth and Leitch (195) methylated lactose and hydrolyzed the heptamethyl methyllactoside, thus obtaining 2,3,5,6,-tetramethyl galactose as expected (assuming the 1,4-lactone structure for the hexoses) and a trimethyl glucose which they proved, by elimination of the other possibilities, to be 2,3,6,-trimethyl glucose. This proved that it is the 5 carbon atom that is involved in the linkage of glucose to galactose. They give the following linkage for lactose, which is generally accepted as correct:



Fischer in his work on enzyme specificity (100) (196) found that an enzyme which would hydrolyze lactose would also hydrolyze a β-methylgalactoside, but not an α-methylgalactoside. On the other hand an enzyme which would hydrolyze an α-galactoside, but not a β-galactoside, would not hydrolyze lactose. Hence he concluded that lactose is a β-galactoside. Perkin (197) gives the evidence of the magnetic rotation of lactose to prove that there is a partial change from an α-glucose-β-galactoside to a β-glucose-β-galactoside when lactose is dissolved in water.

ISOMERISM AND EQUILIBRIA

The earliest statements indicating the existence of more than one form of lactose are those of Erdmann in 1855 (198), who noted the mutarotation of lactose solutions, and those of Dubrunfaut (199), who in the following year recorded that a solution of lactose, saturated at 10° and allowed to evaporate at ordinary temperatures, did not begin to separate out crystals till the concentration had increased from 14.58 per cent to 21.64 per cent. Erdmann (200) and Schmöger (201) worked independently on the mutarotation problem, and established the value of the specific rotation of the hydrate in the vicinity of 84°, that of the anhydride in the vicinity of 39°, and the final value for both in the vicinity of 55°. Schmöger (202) also established the facts that the evaporation of a lactose solution at 100° gives anhydrous lactose, but that the hydrate does not lose water at 100°. Urech (203) measured the rate of change of rotation of lactose to the final value and noted the tremendous accelerative effect of ammonium hydroxide and the milder effect of hydrochloric acid on the rate of change. Tanret (204) apparently was the first to recognize the existence of definitely different modifications of lactose. He listed three forms, − α with $(\alpha)_D = +88°$, β with $(\alpha)_D = +54.6°$, and γ with $(\alpha)_D = +34.5°$. He later concluded that his β form was a mixture of the other two forms (205).

The most extensive investigations on the mutarotation of lactose are those of Hudson (206) and of Gillis (207). They

both considered the closely related subject of solubility relations. The following discussion is based mainly on their results and conclusions.

If a lactose solution is allowed to crystallize at a temperature below 93°, there is obtained the ordinary lactose—that is, the hydrate, $C_{12}H_{22}O_{11} \cdot H_2O$. This is the stable form at ordinary temperatures, since the other forms change below 93° in the presence of water to the hydrate. If the crystallization takes place above 93°, the crystals are anhydrous β-glucose-β-galactoside, or, more briefly, β-lactose (anhyd.). If the more nearly perfect crystals are selected and washed successively with hot glycerine, hot 95 per cent alcohol, and ether, a product of a high degree of purity is obtained. β-lactose (anhyd.) is the stable form at temperatures above 93° as proven by its method of preparation and by the fact that the solid hydrate in the presence of water changes to the β-anhydride above 93°. This form may be preserved indefinitely at ordinary temperatures in the absence of water. If the hydrate is dehydrated at any convenient temperature above 65°, α-lactose (anhyd.) is obtained. This too is not changed in the absence of water, but in the presence of water it changes to the hydrate below 93°, to the β-anhydride above 93°. The α-anhydride heated in contact with a few crystals of the β-anhydride remains unchanges. α-lactose (anhyd.) may be considered metastable at all temperatures in the presence of water. It is α-glucose-β-galactoside, being the analog of α-glucose from the standpoint of molecular rotation and structure.

Since lactose begins to decompose perceptibly at 130°, it is not possible to determine the transition point for α-anhydride $\rightleftarrows$ β-anhydride by ordinary methods. Gillis used the Soch method (208) of determining melting points, which consists of plunging capillary tubes containing the substance into molten baths the temperature of which is close to the expected melting point. He found the melting point of the β-anhydride to be 252.2° and that of the α-anhydride 222.8°. Hence, the transition point is not below the melting point. By the same method, he found the melting point of the hydrate to be 201.6°.

Hudson concluded from his equilibrium studies that the transi-

tion point between the hydrate and the β-anhydride was at 93°. Gillis, using the solubility figures for the β-anhydride and the hydrate and the integrated form of the van't Hoff formula, $\text{nl}C = -Q/2T + C$, plotted log C against $10^3/T$. He obtained an intersection of the two curves at a point between $10^3/T = 2725$ and 2730 corresponding to 93.3° and 93.8°. He therefore assumed 93.5° as the probable transition temperature. Gillis concluded that, since the α-anhydride is always formed on dehydration of the hydrate, the hydrate itself must be of the α form. This is contrary to Hudson's assumption that aldose hydrates have a terminal group-$CH(OH)_2$ which would not permit them to exhibit an α-β asymmetry. This will be mentioned again, but the point of interest here is that, according to Gillis, 93.5° is both a transition and a dehydration point.

The specific rotation of the hydrate is initially about +89°, that of β-lactose initially about +35. After 24 hours, the specific rotation has changed in both cases to the same value, +55.5°. The solution now contains an equilibrated mixture of the two forms. The rates of change of the rotatory powers of both forms correspond to incomplete monomolecular reactions and the constants are the same. Hudson determined the ratio of initial to final rotation at 25° to be 1.55 for the hydrate and 0.64 for the β-anhydride. Gillis obtained 1.61 and 0.63 respectively, working at 0°. Multiplying the specific rotation of lactose in an equilibrated mixture by each of Gillis's factors gives 89.4° as the specific rotation of the hydrate form and 35.0° for the β-anhydride form. Furthermore, by determining the ratio of initial to final rotation for known mixtures of the two forms and plotting composition against this ratio, there is obtained a straight line. At the point where the ratio equals unity—that is, equilibrium,—the solution contains 62.25 per cent of its lactose in the β form and 37.75 per cent in the α form, and the equilibrium constant equals 62.25/37.75 or 1.65 (at 0°).

Gillis has constructed the isotherms for the two forms of lactose below, at, and above 93°, and a space model of the pseudo-ternary system involving lactose and water. These will be found in either of his articles referred to. The fact that no solid

β-hydrate has been isolated is taken to prove that its solubility is greater than that of the β-anhydride.

Hudson's theory of mutarotation as applied to lactose is expressed by the relation:

$$\alpha\text{-anhydride} + H_2O \underset{1}{\rightleftarrows} \text{hydrate} \underset{2}{\rightleftarrows} \beta\text{-anhydride} + H_2O$$

He states that equilibrium 1 is quickly established and equilibrium 2 is slowly established in the case of lactose. He admits that the reverse is the case for maltose and offers no explanation for such a striking difference between otherwise analogous equilibria.

Gillis objects to Hudson's theory on account of the difficulty mentioned above and on account of the assumption of $-CH(OH)_2$ as the terminal group of aldose monohydrates, which denies the lactonic structure of hydrates and the asymmetry of the terminal carbon atom. Hudson himself has shown that the rotational effect of the terminal group of aldoses has a definite additive value which is positive or negative depending upon whether the aldose is of the α or β form. This holds for lactose and would seem to prove that the terminal group of the ordinary hydrate was the mirror image of that of the β form and was identical with that of the α form.

Gillis formulates the lactose equilibria as follows:

$$\begin{array}{ccc} \alpha\text{-anhydride} + H_2O & \leftrightarrows & \alpha\text{-hydrate} \\ \uparrow\downarrow & & \uparrow\downarrow \\ \beta\text{-anhydride} + H_2O & \leftrightarrows & \beta\text{-hydrate} \end{array}$$

The hydration equilibria become established almost instantaneously and the real cause of mutarotation, according to Gillis, lies in the slower establishment of the equilibria:

$$\begin{array}{ccc} \alpha\text{-anhydride} & & \alpha\text{-hydrate} \\ \downarrow\uparrow & & \downarrow\uparrow \\ \beta\text{-anhydride} & & \beta\text{-hydrate} \end{array}$$

Gillis points out that his theory does not require the assumption of speeds of different orders for analogous reactions of different sugars.

Leighton and Peter (209) have obtained a definite supersaturation curve for the equilibrium mixture of lactose. This curve lies about 30° below the saturation curve of the equilibrium mixture. In the metastable area between these curves, crystallization can be induced only by the introduction of a sufficient number of suitable nuclei, and it is possible to obtain a slow crystal growth without producing general crystallization. In the labile area below the supersaturation curve, a general crystallization will be produced by any nuclei, but without nuclei crystallization will not necessarily take place. It is possible to carry lactose solutions well into the labile area with the separation of ice alone.

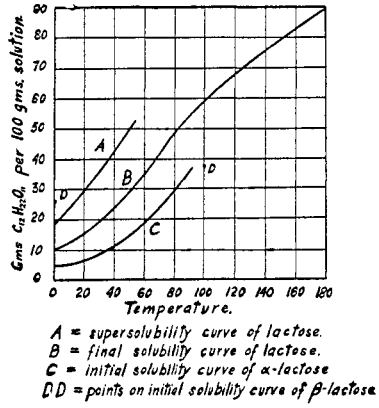


FIG. 1. SOLUBILITY OF LACTOSE

Herewith are included solubility curves constructed on the values of Hudson (206), Saillard (116), and Gillis (210), and the supersaturation curve just discussed.

GENERAL PHYSICAL DATA

Lactose hydrate is insoluble in 95 per cent ethyl alcohol, methyl alcohol, and ether, soluble to about 2 per cent in pyridine (211), soluble somewhat in warm acetic acid, either concentrated or dilute, from which it crystallizes unchanged on cooling (212). It dissolves in about 6 parts of cold and 2.5 parts of hot water and crystallizes therefrom in rhombic prisms. The taste of lactose is less sweet than that of sucrose and, on account of the low solu-

bility and the hardness of its crystals, it produces in the mouth a sensation like that produced by sand.

The ratio of the axes of the crystals is given by Schabus (213) as a:b:c = 1:0.6215:0.2193, and by Traube (214) as a:b:c = 1:0.3677:0.2143. Traube also gives 109° 47′ as the value for β. The cubical expansion coefficient of lactose is 0.00911 per degree between 0° and 100° (215).

The values for the heat of combustion of lactose show so much

TABLE 2

Heat of combustion of lactose

HYDRATE		β-ANHYDRIDE		AUTHORITY
cal./gram	*Cal./mol.*	*cal./gram*	*Cal./mol.*	
3945	1420	4162	1423	Van Rechenberg (216)
3663		3877	1326	Stohmann (217)
3777				Berthelot and Vielle (218)
3724		3920		Gibson (219)
3951	1351	3737	1345	Stohmann and Langbein (220)
3737				Emery and Benedict (221)
3953				Karrer (222)

TABLE 3

Heat of solution and transition of lactose. 20°C.

	HYDRATE	α-ANHYDRIDE	β-ANHYDRIDE
	cal./gram	*cal./gram*	*cal./gram*
Initial heat of solution	−12.0	+7.3	−2.3
Final heat of solution	−11.4	−7.9	−2.7
Temperature coefficient of heat of solution	0.1		
Heat of transition to β-anhydride	+1.0	+1.0	
Heat of transition to equilibrium mixture		+1.03	

variation that the principal ones are given in table 2. The heat of formation of the hydrate is given as 535.6 Cal./mol., of the β-anhydride 610.8 Cal./mol. (220). The specific heat of the hydrate is 0.299, of the β-anhydride 0.2895; the molecular heat of the solid hydrate is 107.6 cal., of the solid β-anhydride 99 cal.; the apparent molecular heat of either form when dissolved in water is 147 cal. (223). Table 3 (224) gives values for heats of solution and transition of the various forms of lactose.

The earliest determination of the specific gravity of lactose was probably that of Lichtenstein (12), who found it to be 1.543. This value is surprisingly close to that of Fleischmann and Weigner (225), who calculated from the equation given below the specific gravity of "liquid" lactose and found it to be 1.5453. These workers extended the tables of Schmöger (201b) for observed specific gravity of lactose solutions up to concentrations of 69 per cent and derived the equation,—$S_x = 0.9982 + 3.7585\,(10^{-3}X) + 1.1284\,(10^{-5}\,X^2) + 5.8405\,(10^{-8}X^3)$, in which S_x is the specific gravity of a solution containing X per cent lactose. $S_{100} = 1.5453$. From a comparison of specific gravity

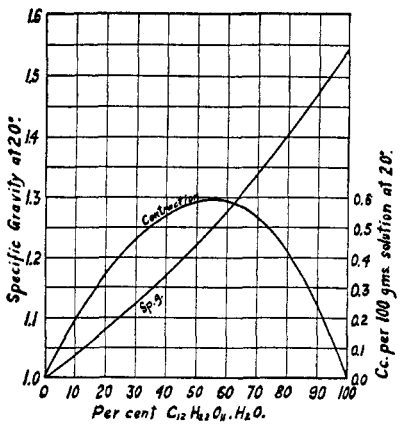


FIG. 2. SPECIFIC GRAVITY AND CONTRACTION OF LACTOSE SOLUTIONS

values calculated on the assumption of no contraction and the actual values obtained, they calculated the contractions of lactose solutions of various concentrations and derived the equation: $K = 0.34382X - 0.0031819X^2 - 8.692$, in which K is the contraction in cc. for 100 gms. of solution at 20° containing X per cent lactose. The maximum is 0.593 cc. per 100 grams solution and occurs at a concentration of 54.03 per cent lactose. A solution of this concentration contains 18 mols H_2O for each mol of $C_{12}H_{22}O_{11}$. The molar volume of hydrated lactose is 235.2. It has been pointed out (226) that this value is equal to that of the volume of 12 mols of water as ice.

The accompanying plots of specific gravity and contraction are constructed on the values of Schmöger (201b) and Fleischmann and Weigner (225).

MANUFACTURE

The manufacture of lactose is usually carried out in connection with cheese manufacture, since the whey which is the source of lactose is a by-product of the cheese industry. Furthermore, the whey is perishable in so far as its lactose content is concerned and is too bulky to ship profitably. Any scheme for lactose utilization must take these facts into account.

Up to about 1880, lactose was produced only in Canton Luzern, Switzerland. Shortly after this, factories were established in Germany and the United States. In 1893, one factory in New York reported the production of over 250,000 pounds. In 1914 there were produced in the United States 3,500,000 pounds, distributed among sixteen factories. The production for 1922 had dropped to 2,190,000 pounds. Domestic production is somewhat irregular, due to trade conditions and the limited demand.

The original Swiss method (227) was to prepare a crude "sugar sand" at the small cheese factories high in the Alps. This was brought to a central point for refining. The whey was evaporated over direct wood fires in more or less open huts. Evaporation was continued till the whey was of the consistency of syrup or honey and would fall in sheets, "zu blätteln," from a spoon. It was then run into shallow pans and allowed to crystallize for forty-eight hours. The more rapid the cooling and the lower the final temperature, the better the quality and the higher the yield of the sugar sand. Care was taken that the crystals should be neither too fine nor too coarse; in the first case, too much sugar would be lost during washing; in the second case, the impurities could not be effectively removed. The oily mother liquor was poured off and concentrated further to obtain a second crop of crystals. The crystals, after removal of the mother liquor, were stirred with water two or three times, the first two washings being used for hog feed, the last being added to another batch of whey. The sugar was then allowed to drain for a time and sacked for shipment to the refinery.

This process gave about 2.0 to 2.5 per cent of the weight of the whey as crude sugar, or 1.2 to 1.5 per cent as refined lactose. If the lactic acid present was neutralized with lime before evaporation to prevent inversion, yields of lactose as high as 4 per cent of the whey were often obtained. Another method of improving the yield was to dialyze out the salts from the first mother liquor and then add the liquor to a new batch of whey. Concentration by freezing was sometimes used to save fuel.

In the refinery the sugar sand was dissolved in water at 75° and the solution heated to boiling. About one per cent of alum was added and the liquid gently boiled. The scum of impurities was removed and the solution run through a charcoal filter. It was then evaporated "zu blätteln," run into a copper-lined wooden vat, and allowed to crystallize on a raised network of sticks. After four or five days the crust of crystals on the surface would begin to sink and the crystallization was considered complete. The better grade of crystals were shipped, the poorer were purified again.

The more modern methods for lactose manufacture use to a considerable extent the equipment of the cane and beet sugar industry. Lactose solutions are particularly susceptible to the action of heat and the use of a vacuum pan for evaporation is of great assistance in obtaining a white product.

The modern process may be outlined as follows (228): Any butterfat present is removed by a separator. Albumen may be removed by heating nearly to boiling after addition of acetic acid, or it may be left in the whey till later. The whey is then evaporated under vacuum at 60° to 70° until it contains about 60 per cent solids. It is then run into iron crystallizers holding about 174 gallons. These crystallizers have water jackets in which cold water is circulated. For ten hours, or often longer, the mass is frequently stirred to equalize the temperature. After about 24 hours it has become a thick, coarse-grained, yellow mush. This is centrifuged and yields about 3.8 per cent of the weight of the original whey as wet raw sugar containing about 88 per cent lactose.

The mother liquor is then boiled by direct steam to coagu-

late the albumen, which is removed. The clear liquid is then concentrated in vacuo as before and more lactose crystallized and centrifuged out. About 0.5 per cent additional yield of sugar is obtained, making a total yield of about 4.3 per cent of the weight of the whey. The second mother liquor is mostly run to waste or worked up for fertilizer, though efforts have been made to utilize it for fattening hogs, for the preparation of lactic acid, and as a basis of a food product similar to meat extract.

For refining, the raw sugar is dissolved in water at 50° till the solution registers 13° to 15° Bé., or contains 24 to 27 per cent of the sugar. Powdered bone black and acetic acid are added. It is then heated nearly to the boiling point and a small quantity of magnesium sulfate added. The liquid is then boiled till it is decolorized. Considerable foaming occurs and a flocculent precipitate of protein and phosphates separates out. This precipitate is filterpressed, washed, and treated with sulfuric acid to produce a fertilizer high in nitrogen and soluble phosphoric acid. The filtrate is evaporated in vacuo until it registers 35° Bé., equivalent to about 65 per cent solids. It is then crystallized and centrifuged.

Two more crops of crystals are obtained by further evaporation. The refining process is repeated till the desired quality of lactose is obtained. The purified product is dried in a rotating air dryer till the particles will not cohere when pressed between the hands. It is ground in an edge-runner mill and sifted to such a fineness that particles cannot be felt between the fingers. The weight of the finished lactose should average 2.5 per cent of the weight of the whey. The remainder of the lactose of the whey is partly lost by fermentation and by inversion and partly held in solution in the various discarded mother liquors. The other constituents of the whey have a considerable inhibitory action on the crystallization of the lactose.

Several other methods of obtaining lactose from whey have been suggested, among which may be mentioned the evaporation of whey to dryness and the extraction of the lactose with water (229); the evaporation of whey on kieselguhr or other absorbent material with subsequent leaching of the lactose (230); and

evaporation with moderate heat, extraction of the albumen with a limited amount of water, and finally extraction of the lactose (231). Due to interest in soluble milk albumen, it is possible that existing factory methods for obtaining lactose from whey will be largely modified to include production of albumen.

QUALITY, SPECIFICATIONS, AND USES

Examination of seventeen samples of domestic lactose by England (232) indicated that the following specifications for lactose are reasonable:

> Sugar of milk of acceptable quality must be a fine, white, dry, odorless powder of not less than 99.7 per cent strength by the polariscope, containing not more than 0.020 per cent nitrogen, not more than 0.020 per cent fat, and yielding not more than 0.050 per cent ash. It must comply with the U. S. P. heavy-metals test and be neutral to litmus paper. A ten per cent solution must be odorless, colorless, and free from mechanical impurities.

For bacteriological use, lactose must satisfy certain additional tests (233). It should prove to be free from alcohol by the iodoform test and contain not more than 0.15 per cent moisture. A 10 per cent solution should show not more than a negligible turbidity when tested for sulfates with barium chloride or for chlorides with silver nitrate. A 10 per cent solution sterilized for thirty minutes at 120° should show a pH value not greater than 4.0 and should remain acid on cooling. *d*-glucose should be absent as proven by failure to produce acid with Bacterium typhosi B. or to produce gas with yeast. Incubation of a sterilized solution should show no growth.

The literature on the relative value of lactose and other sugars in nutrition is in such an unsatisfactory state that few definite well-founded statements can be made. Instinctively, we are led to believe that because lactose is the sole sugar occurring in milk it must possess some unique characteristic which makes it peculiarly valuable in nutrition. Lactose does differ in some respects from each of the other sugars, but, from the standpoint of nutrition, it is difficult to decide what property or properties

give it any advantage over other sugars. The fact that it is fermented only by certain types of organisms is as good a guess as any. On the other hand, it has been seriously argued (234) that sucrose should be substituted for lactose in infant feeding for such reasons as the non-identity of the lactose of bovine and of human milk, the impurities in commercial lactose, the great danger from the large quantities of lactic acid formed from lactose, and the purgative effect of lactose in large daily doses (235). Another writer (236) advocates for addition to artificial diets for children a mixture of 40 per cent lactose, 40 per cent sucrose, and 20 per cent maltose. The personal tolerances and other idiosyncrasies of the subjects of experimentation are probably as important factors in the reaching of such conclusions as the scientific knowledge and personal prejudices of the experimenter. The fact remains that large quantities of lactose are consumed in milk and milk products, that lactose is used in considerable amounts in proprietary infant foods, and that it is prescribed quite generally by physicians for modifying cow's milk for infant feeding.

If lactose or milk be fed to animals or human subjects in such quantities that the lactose is not all split in the small intestine, the excess may be utilized by lactose-fermenting bacteria, of which B. coli communis and Lactobacillus acidophilus are the chief intestinal representatives. The effect of lactose on the intestinal flora has been extensively investigated by Rettger and his associates (237). They find that feeding lactose or dextrin will change the flora of the large intestine from a putrefactive type to a fermentative type and that the fermentative organisms are almost entirely Lactobacillus acidophilus. The change is considerably accelerated if cultures of this organism be fed together with the sugar. None other of the common sugars produces this result. This is explained by observations that lactose and dextrin alone of the saccharides used succeed in reaching the ileocaecal valve before being absorbed; hence these are the only saccharides capable of stimulating the multiplication of this organism. The frequently advanced explanation of the disappearance of the putrefactive organisms when lactose is

fed—namely, that Lactobacillus acidophilus produces a high acid concentration in the intestine that the putrefactive organisms cannot survive but that it can survive itself—is disputed by Rettger, who failed to find any appreciable change in the H-ion concentration of the intestinal contents after implantation of Lactobacillus acidophilus. Very beneficial results are being obtained by the use of cultures of Lactobacillus acidophilus in milk for the treatment of autointoxication. It should be pointed out that, while Lactobacillus acidophilus is a normal inhabitant of the intestine and can be easily maintained there, Lactobacillus bulgaricus, in spite of the contrary assumption, cannot be implanted and maintained in the intestine. The successful therapeutic use of cultures of this organism has been shown to be due to the stimulation of Lactobacillus acidophilus by the increased quantities of lactose fed with the bulgaricus cultures.

The therapeutic value of lactose is not confined to the action just discussed but includes its pronounced laxative and diuretic effects (238). It is often somethat difficult to separate sharply the laxative effect from the effect of the change of intestinal flora. Both the laxative and the diuretic effects are due probably to the dehydrating action of lactose.

In addition to the uses already mentioned, lactose is used by confectioners in certain types of candies; by manufacturing pharmacists as a sweetener, diluent, and vehicle in the preparation of medicines in tablet form; and by the manufacturers of certain liqueurs on account of the frosty appearance produced by crystallization of lactose on the inside of the bottles. The total consumption of lactose in this country is far less than the amount that could be produced from our dairy wastes; consequently, it is demand rather than potential supply that determines the extent of manufacture of this sugar.

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