

THE DETERMINATION OF LACTOSE IN MILK.

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In this paper we describe two methods for the determination of the sugar in milk—a titration method and a colorimetric method. Of the two procedures we believe the titration to be the more accurate. The colorimetric method has, however, the advantage that by means of it a very large number of determinations can be made more or less simultaneously.

I. Titration Method.

In the sugar titration method of Folin and McEllroy 40.4 mg. of anhydrous lactose have the same reducing value as 25 mg. of dextrose. The lactose value was determined by us because it was thought that the method might prove quite as serviceable for the titration of the lactose of milk as for the titration of the dextrose of urine. Since albumin does not interfere with that titration it was hoped that the lactose of milk could be titrated directly, that is without any preliminary preparation of protein-free filtrates. A remarkably simple method for the determination of the sugar in milk would thus be secured. Our conjecture turned out to be correct, for we have encountered no difficulties in titrating the lactose of milk without first removing the protein and the fat. By means of large numbers of check determinations, using protein-free filtrates and determining the sugar by titration, by the polariscope, as well as colorimetrically, we have satisfied ourselves that the preliminary removal of the protein materials is superfluous. Omitting the preparation of protein-free milk filtrates eliminates not only the most tedious feature of the older methods for titrating the sugar in milk, but it also does away

with the cumbersome "corrections" for the volume occupied by the protein-fat precipitates.

Because of the extreme variations in the sugar content of urine it seemed desirable to be able to titrate it without preliminary dilution. In the case of milk the circumstances are entirely different, for the variations in the sugar content of this secretion are relatively very small, and a *suitable* preliminary dilution, without any uncertainty and without any approximate preliminary sugar titration, can be made. It has therefore seemed to us rather better to dilute milk before titrating, though the titration can be made quite well without any such dilution. We recommend a dilution of 1:4 (25 cc. in a 100 cc. flask) for cow's milk and 1:5 (5 cc. in a 25 cc. flask) for mother's milk. In emergencies, as when the quantity of human milk available for the sugar titration is very small, a dilution of 1:6 (2 cc. added to 10 cc. of water) can be used.

The titration is made as follows: Into a large test-tube introduce 2.8 to 3.4 cc. of the diluted milk (that is, nearly the full amount expected), 5 cc. of a 6 per cent copper sulfate solution, a pebble (to prevent bumping), and 4 to 5 gm. of a dry salt mixture (made by mixing, in powdered form, 100 gm. of disodium phosphate, 60 gm. of anhydrous sodium carbonate, and 30 gm. of sodium or potassium sulfocyanate). Shake, and boil gently for 4 minutes before adding any more milk. At the end of this time add more milk (0.02 cc. to 0.10 cc., depending on the amount of blue color remaining) and boil again. After each addition of milk (except the first) boil 1 minute. The total boiling period should be 5 to 7 minutes.

For the measurement of the milk we recommend the 5 cc. burette and other accessories, mentioned in the preceding paper in connection with the titration of sugar urines.¹

Calculation: 4.04 multiplied by the degree of dilution (4, 5, or 6) and divided by the titration figure, gives the per cent of lactose present.

¹ We have up to date made over 200 sugar determinations by this new titration method—most of them on human milk. This work has developed into a relatively extensive study of the sugar content of human milk and will be published later.

II. Colorimetric Method for the Determination of Lactose.

Dehn and Hartman² have already described a colorimetric picrate method for the determination of the sugar in milk. Our procedure is materially simpler, and it gives, we believe, as accurate results as it is possible to obtain by a colorimetric picrate method.

The method is as follows: With an accurate 2 cc. pipette transfer 2 cc. of milk to a 100 cc. volumetric flask previously half filled with saturated picric acid solution. Fill to the mark with saturated picric acid solution, shake, and filter. Transfer 5 cc. of the filtrate to a large test-tube or 100 cc. volumetric flask, add 15 cc. of saturated picric acid solution and 3 cc. of 20 per cent sodium carbonate solution. Mix and place in a boiling water bath for 15 minutes. Cool and dilute to a volume of 100 cc. The standard consists of a solution of lactose in saturated picric acid solution. For use with cow's milk this solution should contain 5 mg. of lactose in 20 cc. For use with human milk it is necessary to employ two standards; namely, one containing 5 and the other containing 7 mg. of lactose in 20 cc. To 20 cc. of the standard add 3 cc. of the sodium carbonate solution and heat simultaneously with the milk filtrate. Cool, dilute to 100 cc., and compare the colors in the usual manner in a suitable colorimeter.

According to our experience accurate results cannot be uniformly obtained except by heating the standard simultaneously with the unknown and it is therefore absolutely necessary to prepare a new standard for each set of determinations. Nor can the color values of the standard and the unknown be more than 20 per cent apart.

In the following table are given a few figures representing parallel determinations of lactose in milk made by the colorimetric and by the titration methods.

² Dehn, W., and Hartman, F. A., *J. Am. Chem. Soc.*, 1914, xxxvi, 404.

Determinations of Lactose in Cow's and in Women's Milk.

No.	Lactose.	
	Colorimetric method.	Titration method.
	<i>per cent</i>	<i>per cent</i>
Cow 57.....	4.42	4.41
" 41.....	4.08	4.30
" 37.....	4.13	4.22
" 22.....	4.54	4.46
" 39.....	4.87	4.84
" 45.....	4.51	4.54
" 31.....	4.21	4.23
" 31.....	4.37	4.45
" 44.....	4.60	4.50
" 38.....	4.64	4.68
Mother 1.....	3.08	3.10
" 8.....	5.73	5.75
" 9.....	5.07	5.05
" 10.....	4.79	4.83
" 12.....	6.73	6.63
" 13.....	6.64	6.53
" 16.....	5.87	5.87
" 17.....	6.37	6.40

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