	Gram.
Seed weighed	0.1244
In seed nitrogen	0.0038
Dry matter	0.4768
Nitrogen found	0.0056

Pot 6.—Same as 5.

	Gram.
Seed weighed	0.1285
In seed nitrogen	0.0039
Dry matter	0.8350
Nitrogen found	0.0074

Pot 7.—Same as 5 and 6.

	Gram.
Seed weighed	0.1175
In seed nitrogen	0.0036
Dry matter	0.9664
Nitrogen found	0.0080

In all these instances growth had stopped before the plants were removed from the pot. The gain in nitrogen without soil infusion was 0.0028 gram; with soil infusion, 0.0018, 0.0035, and 0.0044 gram. Apparently it mattered very little whether the soil infusion was added or not and in all the instances the gain was so inconsiderable as to lie well within the limits of error of the experiments. It would seem therefore that under the conditions employed the cotton plant does not assimilate atmospheric nitrogen.

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DETERMINATION OF LACTOSE IN MILKS BY DOUBLE DILUTION AND POLARIZATION.

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IN volume 6, page 289, of the *American Chemical Journal*, one of us (Wiley) published an article on the determination of lactose in milks by optical methods. The principal novelty in this process was the substitution of mercuric nitrate as the reagent for precipitating proteids in place of the other reagents which had usually been employed for that purpose. By the use of mercuric nitrate in an acid solution, it was shown in that

paper that it was possible to practically throw out all of the proteid dissolved in the milk. Inasmuch as these soluble proteids are optically active, and deflect the plane of polarization in a direction opposite to that produced by lactose, the presence of any notable quantity of them in the solution to be polarized tends to diminish the apparent percentage of lactose present. The reagent proposed, *viz.*, acid mercuric nitrate, when used in the cold and in the quantities specified, produces no inversion effect whatever upon the lactose.

In the paper referred to an arbitrary correction was made for the volume of the precipitate produced and this was fixed at two and five-tenths cc., when approximately sixty cc. of milk were used in a 100 cc. flask.

This method of estimating lactose on account of the ease with which it can be operated and its accuracy has been generally adopted by chemists. Attention has been called, however, to the fact that the arbitrary correction allowed for the volume of the precipitate is too small.[1]

Theoretically, it is evident also that the arbitrary correction admitted is too small except in cases of well-skimmed milk. In order to eliminate this arbitrary factor from the method, we undertook a series of experiments to determine the actual percentage of sugar and the proper correction to be allowed for the volume of the precipitate by the method of double dilution and polarization originally proposed by Scheibler for sugar solutions, and suggested by Bigelow and McElroy for use in the polarization of milk sugar.[2] The results of our determinations are extremely satisfactory, and show that the volume which is occupied by the precipitate in a milk varies from two and a half cc,, in the circumstances mentioned above, to six cc., according to the richness of the milk in fat. It appears, however, that this correction is less in quantity than the apparent combined volume of the fat and albuminoids which may be safely assumed to be one cc. for one gram.

All the flasks which were employed in the determinations were carefully calibrated, and the instrument used was the new triple-field shadow polariscope, made by Schmidt and

[1] *Analyst*, **12**, 64; **20**, 126.

[2] This Journal, **15**. 694.

Haensch, which enables readings to be accurately made to within 0.05 per cent. All readings were made in duplicate by each of us and entered before comparisons were made, and in the polarizations given in the table the means of these four readings, which never differed by more than one-tenth per cent., are given. The polarizations were made on the contents of a 100 and a 200 cc, flask, after clarification of the milk by means of acid mercuric nitrate. In each case, double the quantity of the normal weight of milk for the instrument used was taken. The readings were calculated by the formula given by Scheibler, which requires that the reading obtained from the solution in the large flask be multiplied by two and subtracted from the reading obtained in the small flask. In all cases, in order to secure greater accuracy, our readings were made in a tube 400 mm. in length. Therefore, the data obtained in reading the solution in the small flask were divided by four in order to obtain the apparent percentage of sucrose.

The application of the formula given by Scheibler does not give absolutely accurate results. The true polarization in any given case is calculated according to the following scheme:

Let x equal the volume of the precipitate and y the correct reading. Let a equal the reading obtained from the solution in the small flask and b equal the reading of the solution from the large flask. We then have

$$200 - x : 100 - x :: a : b$$

$$200\,b - bx = 100\,a - ax$$

$$ax - bx = 100\,a - 200\,b$$

$$x = 100\,\frac{(a - 2b)}{a - b} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$100 - x : 100 :: y : a$$

$$100 - 100\,\frac{(a - 2b)}{a - b} : 100 :: y : a$$

$$100\left(a - \frac{a\,(a - 2b)}{a - b}\right) = 100y$$

$$y = a - \frac{a\,(a - 2b)}{a - b}$$

$$y = \frac{a^2 - ab - a^2 + 2ab}{a - b} = \frac{ab}{a - b} \qquad \ldots\ldots\ldots\ldots (2)$$

The rule derived from formula No. 2 is as follows:

The true polarization, as determined by double dilution, is found by dividing the product of the two readings made from the solutions in the large and small flasks by their difference.

In order to test the accuracy of the method, known volumes of insoluble material, as, for instance, quartz sand, were added to the flasks in order that the volume of the precipitate might be increased by a certain definite amount. The determinations were also made on the whole milk as purchased, on the same milk deprived of the most of its cream and on the cream thus secured. In all cases the results obtained were perfectly satisfactory.

Blyth has lately described a method of precipitating the casein with acid and of washing the precipitate free of sugar on a filter and polarizing the filtrate.[1] The percentage of milk sugar in the mixed filtrate and washings is about one and the polariscopic reading should be corrected for that degree of dilution. This method evidently is better suited for preparing milk whey for the gravimetric estimation of the sugar by copper, since it takes no account of the albumens still in solution and serving to a certain extent to counteract the polarizing power of the lactose.

In the presence of sucrose he proposes to estimate its quantity from the property possessed by citric acid of inverting the sucrose and leaving the lactose unchanged. Raumer and Späth[2] suggest that the polarization of milk should be preceded by boiling, since it is probable that the lactose may exhibit birotation. The data which they adduce, however, are far from convincing, since after the boiling they clear the mixture with lead subacetate and it has been shown that this reagent does not remove all the proteids. The deficit in rotation is therefore probably due to the residual soluble left-handed proteids. They further suggest that the presence of a dextrinoid body, as indicated by Ritthausen[3] may serve to increase the actual rotation of the milk sugar. In the samples which showed the apparent

[1] *Analyst*, 20, 122.
[2] *Ztschr. angew. Chem.*, *1896*, 72.
[3] *J. prakt. Chem.* (2), 15, 348.

increase, however, they made no attempt to prove the presence of the alleged disturbing dextrin.

There seems to be no just reason, therefore, for insisting on the slow and tedious gravimetric method when a quick and accurate optical method is at hand.

Inasmuch as the time required for carrying out the method of double dilution and polarization is scarcely any longer than that required for a single polarization, it is recommended that it be done in all cases, instead of correcting the results of a single polarization by any arbitrary factor. When the determination is conducted as suggested, the analyst has at hand an easy, rapid, and accurate method of estimating milk sugar in milk, which is as desirable in all respects as any gravimetric method whatever. The data obtained are given in the accompanying table.

POLARIZATION OF MILK BY DOUBLE DILUTION.

No.	Per cent. fat.	Polarization in 200 cc. flask.	Polarization in 100 cc. flask.	Apparent per cent. lactose.	True per cent. lactose.	True volume in 100 cc. flask.	Volume of precipitate.
1	....	9.37	19.26	4.82	4.56	94.4	5.6[1]
2	....	9.59	20.33	5.08	4.54	88.8	11.6[2]
3	....	9.36	19.20	4.80	4.57	95.0	5.0[3]
4	....	9.60	20.25	5.06	4.56	89.7	10.3[4]
5	2.9	10.15	20.84	5.21	4.95	94.8	5.2
6	4.8	10.31	21.21	5.30	5.00	94.5	5.5
7	3.1	9.49	19.41	4.85	4.64	95.7	4.3[5]
8	4.0	10.01	20.45	5.11	4.90	95.9	4.1
9	1.4	9.44	19.26	4.82	4.63	96.1	3.9[6]
10	5.5	11.05	22.68	5.67	5.38	94.8	5.2
11	4.4	9.57	19.47	4.87	4.71	96.5	3.5[7]
12	2.0	9.75	19.93	4.98	4.77	95.8	4.2[8]
13	17.6	8.72	19.13	4.78	4.01	82.4	17.6[9]

Summary of Method.—For the scale of the instrument used, 32.91 grams of pure lactose in 100 cc. give a reading of 100. This number is derived from the following data: For sucrose concentration twenty-five grams in 100 cc., $[\alpha]_D^{20^\circ}=66.37$.

[1] Without sand.
[2] With five cc. quartz sand.
[3] Without sand.
[4] With five cc. quartz sand.
[5] Same as No. 6, after separation of cream.
[6] Same as No. 8, after separation of cream.
[7] Whole milk.
[8] Skimmed milk.
[9] Cream.

For lactose, thirty-three grams in 100 cc., $[\alpha]_D^{20^\circ} = 52.53.$; then $66.37 : 52.53 :: x : 26.048$, whence $x = 32.91$. The temperature of the working room should be kept at about 20°, since the rotatory power of lactose diminishes in a small degree as the temperature rises. Double the quantity mentioned, *viz*,, 65.82 grams of milk are placed in a 100 cc. flask, clarified with mercuric nitrate solution, the volume completed to the mark, the contents of the flask well shaken, poured upon a filter, and the filtrate polarized in a 400 mm. tube. A similar quantity of the milk is placed in a 200 cc. flask and subjected to the same treatment. The polarimetric data obtained are used for calculating the true volume of liquid in the flask and the true percentage of lactose and the true volume occupied by the precipitate, in accordance with the rule already given, or with sufficient accuracy by Scheibler's formula. The acid mercuric nitrate solution is prepared as follows:

Dissolve mercury in double its weight of nitric acid, specific gravity 1.42, and add to the solution five volumes of water. This solution is more dilute than the one recommended in the original paper, it having been noticed that a stronger solution colors the precipitated proteid matter slightly yellow (xanthoproteic reaction). Ten cc. of the reagent are to be employed instead of two, as directed for the stronger solution. In preparing the solution of milk in the 200 cc. flask it may be necessary at times to use more than this quantity of the acid mercuric nitrate in order to secure a filtrate entirely free of turbidity.

An inspection of the data in the table shows a general agreement between the volume of the precipitate found and the percentage of fat in the sample with the exception of one instance, *viz*., No. 11. It is evident that in solutions so dilute, a slight variation in the volume has a very small influence on the percentage of sugar found. An error of 0.05 degree in the reading of the dilute solution (200 cc. flask) makes an error of 0.05 per cent. in the result. The error due to one cc. of the precipitate in the dilute solution is approximately 0.05 per cent. It is therefore evident that with proper care the percentage of sugar can be determined to within one-tenth per cent. by the polarimetric method and this is entirely sufficient for all practical purposes.