NITRATES AND NITRITES IN MILK.

By G. D. ELSDON, B.Sc., A.I.C., AND JOHN A. L. SUTCLIFFE, A.I.C.

A PORTION of the matter contained in the present communication has received lately a considerable amount of attention. Our results, however, are in some respects contrary to those obtained by other workers, and we therefore publish them, thinking that they may be of general interest. We suggest also a new method for the determination of nitrates in milk when present in any considerable quantity, and which is applicable in the presence of nitrites.

As milk serum must be used in the processes for the estimation of nitrates in milk, we here describe the method of preparation we have adopted.

Preparation of Milk Serum.—We find that the best results are obtained by the method suggested by Tillmans and Sutthoff (*Zeitsch. Untersuch. Nahr. Genussm.*, 1910, 20, 49; ANALYST, 1910, 35, 434), a slight modification being made for the sake of convenience and rapidity. The reagent consists of a 2·5 per cent. solution of mercuric chloride in 1 per cent. w/v hydrochloric acid, and we proceed as follows : Twenty-five c.c. of milk are mixed with 25 c.c. of the reagent, and after well mixing, the whole is poured on to an 11 cm. filter-paper and 25 c.c. of filtrate collected. The rate of filtration may be hastened very considerably by warming the milk before adding the reagent, but in this case the filtrate obtained is not quite so clear. We prefer to work in the cold.

The Detection of Nitrates in Milk.—Since milk is normally free from nitrates, their presence affords strong presumptive evidence of the addition of water, and therefore several methods have been suggested for their detection. The method that seems to have been most favoured is that which depends upon the blue coloration produced by a solution of diphenylamine sulphate in concentrated sulphuric acid. We have found this test to be both delicate and easy of application, and we therefore recommend it for the detection of nitrates; but as the reaction is also given by nitrites, it cannot be used in the presence of the latter. The solution before being examined for nitrates should always be tested for the presence of nitrites by the Griess-Ilosvay method. If nitrites are found, a special method has to be followed in order to test for nitrates. For this purpose we use the brucine-sulphuric acid reagent, which is described later.

We would here draw attention to the erroneous conclusions of P. Soltsien (*Pharm. Zeit.*, 1906, 51, 765; ANALYST, 1906, 31, 374) concerning the diphenylamine test. He states that nitrous acid not only fails to give the reaction, but actually hinders it, when nitrous and nitric acids are present together. We have found that this is not the case, as nitrous acid not only does give the reaction, but gives a deeper colour in equivalent strengths than does nitric acid, and in a shorter time.

The Diphenylamine Test for Nitrates.—The test is carried out in the following manner, which is a slight modification of the method suggested by Tillmans (*Zeitsch. Untersuch. Nahr. Genussm.*, 1910, 20, 676; ANALYST, 1911, 36, 67): The reagent is prepared by adding 50 c.c. of water to 0·085 grm. of diphenylamine sulphate contained

in a large flask, and gradually adding 450 c.c. of strong sulphuric acid. In performing the test 1 c.c. of the serum of the suspected milk is placed in a test-tube (4 by 0.5 in.), 4 c.c. of the reagent are added, and the whole thoroughly mixed and cooled. The presence of traces of nitrates causes the production of a blue colour.

The Brucine-Sulphuric Acid Method.—In the presence of nitrites this reagent (see below) gives an immediate red coloration, which gradually fades to a pale straw tint in three to six hours if nitrates be absent. In the presence of nitrates and nitrites, however, although the first immediate deep red colour will fade, a reddish coloration will persist for several weeks. To detect nitrates, therefore, in the presence of nitrites it is necessary to compare a solution known to be free from nitrates with the one in question by the method given below under the "Determination of Nitrates."

The Presence of Nitrates in Milk.—Potassium nitrate has been used as a preservative, and also to remove the turnip-like taste. Samples of milk recently examined in this laboratory were found to contain potassium nitrate, the vendor stating in court that it had been added for this latter purpose. It is wise, therefore, to examine for nitrates as a routine test, and this is carried out conjointly with the estimation of fat by the Gerber method. When milk containing either nitrates or nitrites is shaken up with 90 per cent. sulphuric acid, a vivid brown colour is produced—golden-brown in the case of nitrites, somewhat darker in the presence of nitrates. This colour is very easily seen on shaking the milk in the Gerber tube with milks containing as little as 5 parts per 10^6 . When such a colour is noticed, the milk should be further tested.

The Determination of Nitrates in Milk.—Tillmans (*loc. cit.*) recommends his diphenylamine method described above; but we have found that, although this gives good comparative results with such quantities of nitrate as are found in watered milks (1 per 10^6), the method is too delicate, however, for concentrations of nitrate about 2 per 10^6 . As larger quantities than this have occurred in practice when potassium nitrate has been added to milk, a method was required for estimating them in such concentrations.

Sulphuric Acid Method.—Five c.c. of milk are placed in a stoppered cylinder, and 5 c.c. of sulphuric acid (sp. gr. 1.770) are added. The cylinder is then shaken, and the colour produced compared with that given with milks containing known quantities of nitrate treated in a similar manner. This method gives good results in the absence of nitrites; but as nitrates and nitrites are sometimes found together in milk, the following test, which is applicable in the presence of nitrites, is of more general application:

Brucine-Sulphuric Acid Method.—The reagent is prepared by dissolving 0.2 grm. of pure brucine in 75 c.c. of sulphuric acid (sp. gr. 1.82), and adding this to 25 c.c. of water.

In the absence of nitrites, 5 c.c. of the serum (*q.v.*) are placed in a test-tube, and 10 c.c. of the reagent are added. The contents are well mixed, poured into a Nessler cylinder and allowed to stand for half an hour. The colour is then compared with standards prepared in the same manner.

In the presence of nitrites the colour is produced immediately on the addition of the reagent, and then quickly commences to fade owing to the bleaching action of

the nitrites. Such action is apparent even after standing all night, although the total effect is not great. We have found, however, that the bleaching effect due to the presence of nitrites upon the colour produced by nitrates is practically independent of the amount of nitrite present—at least, in quantities between 2 and 40 per 100,000.

In such cases of a mixture of nitrate and nitrite the procedure is as follows: Five c.c. of the serum are placed in a test-tube, and 10 c.c. of the reagent are added. At the same time standards are prepared containing suitable quantities of nitrate, and each containing about 10 per 100,000 of sodium nitrite. The contents of the tubes are poured into Nessler cylinders, and matched any time after three hours.

Reduction of Nitrates to Nitrites in Milk on Keeping.—A short time ago some samples of milk were examined in this laboratory, which, after standing, gave a faint nitrite reaction with the Griess-Ilosvay reagent, about 3 parts per 10^6 of sodium nitrite being found. The following day, repeating the determination, 10 parts per 10^6 were present. We eventually found this phenomenon to be due to the presence of nitrate in the milk, this nitrate being decomposed with the formation of nitrite. On going into the matter further, we found that if potassium nitrate be added to milk, after two days nitrite can be detected in the milk, and the quantity becomes appreciable after another day. In order to follow the course of this decomposition, two milks were prepared containing respectively 100 and 20 parts per 10^6 of potassium nitrate. These were tested for the presence of nitrite on five successive days, with the following results :

	Parts per 10^6 of Nitrite found.				
	1st Day.	2nd Day.	3rd Day.	4th Day.	5th Day.
Milk containing 100 per 10^6 KNO_3 .	0	2	8	25	12
Milk containing 20 per 10^6 KNO_3 .	0	0.2	2	4	0

It will be seen from these results that the proportion of nitrite formed increases to a maximum, and then decreases. The above figures were obtained starting with fresh milk, but similar figures were given by sour milk.

The Detection and Estimation of Nitrites in Milk.—The question of nitrites in milk came into prominence some little time ago in connection with a proprietary milk preservative called "Mystin," upon which a report was issued by G. W. Monier-Williams (*Food Reports*, Local Government Board, No. 17; *ANALYST*, 1912, **37**, 155); G. A. Stokes (*ibid.*, 1912, **37**, 178).

In both these papers it is stated that sodium nitrite interferes with the Hehner test for formaldehyde when present in the proportions found when using "Mystin." We have been unable to confirm these statements. With a Hehner solution, prepared by mixing 1,000 c.c. concentrated sulphuric acid and 25 c.c. of a 5.5 per cent. solution

of ferric chloride, we obtained a distinct purple ring after four hours with a milk containing 1 part per 300,000 of formaldehyde and 1 part per 9,000 of sodium nitrite, which is the strength recommended if using "Mystin" as a preservative. The colour produced was certainly not as bright as in the absence of the nitrite, but it was unmistakable. We have repeated this on several occasions, and always with the same result.

G. A. Stokes (*loc. cit.*) suggests that the presence of the formaldehyde will be detected whilst performing the Gerber test, but this we are unable to do if nitrite be present. We find, however, that the presence of a nitrite is shown by the golden-brown colour produced on shaking the tube, the colour produced being somewhat brighter than that produced in the corresponding manner from a nitrate. This colour is unmistakable.

Estimation of Nitrites.—There are two methods in general use for the estimation of nitrites in small quantities—the metaphenylene-diamine and the Griess-Ilosvay methods. Of these the latter is by far the more delicate, and the colour produced is easier to match. The reagent is made up as follows: 0.1 gram. of α -naphthylamine is dissolved in 20 c.c. of glacial acetic acid, and mixed with 0.5 gram. of sulphanilic acid dissolved in 150 c.c. of warm 6 per cent. acetic acid, the whole being diluted to 300 c.c. with more 6 per cent. acid; 150 c.c. of 60 per cent. acetic acid are added to this. In carrying out the test, 5 c.c. of the milk are placed in a test-tube (4 by 0.5 in.), and 3 c.c. of the reagent are added. Milks containing known quantities of nitrite are treated in the same way. The colour produced is matched with the standards after about one hour. The reaction with sulphuric acid can also be made use of as a quantitative test. The procedure is identical to that given above for the estimation of nitrates.

The Detection and Estimation of Formaldehyde in the Presence of Oxidising Agents.—We have stated that nitrites only interfere slightly with the Hehner test for formaldehyde, and that it is possible to detect quantities greater than 1 in 500,000 even in the presence of quantities of nitrite, such as 1 in 5,000. Potassium nitrate seems to have no effect whatever on the Hehner test as carried out by us, and the same is true of potassium chlorate. In this respect our experience differs from that of Stokes and Monier-Williams (*loc. cit.*). For the estimation of formaldehyde in milk under ordinary circumstances we have found the Shrewsbury and Knapp method to be by far the most delicate and the most accurate of the methods yet suggested, but it breaks down in the presence of nitrite. A modification of this test, suggested by Monier-Williams (*loc. cit.*), in which the nitrite is destroyed by urea previous to the colorimetric estimation, has been found by us to work well, and to give results very near the truth. The presence of potassium nitrate does not affect the ordinary Shrewsbury and Knapp test.

The Preservative Action of Potassium Nitrate and Potassium Nitrite.—In the discussion following the paper by Stokes (*loc. cit.*) surprise was expressed that formaldehyde of a concentration of 1 in 300,000 was sufficient to preserve milk for three days, this opinion being based upon the statement by Stokes that "'Mystin' added to milk in the proportion of 3 c.c. per litre was found to be a fair preservative; thus, after three days, the acidity developed was only equivalent to 0.24 per cent. of lactic

NITRATES AND NITRITES IN MILK

acid, whereas the same milk unpreserved showed an acidity equivalent to 0.69 per cent. of lactic acid." We have experimented with formaldehyde and "Mystin" as preservatives in these dilutions, and have found that practically the whole of the preservative action of "Mystin" is due to the sodium nitrite which it contains. In the following tables some of our results are given; the acidities are expressed as c.c. of $\frac{N}{10}$ alkali, using 10 c.c. of milk. The figures given are acidities so expressed :

Date.	Control.	With CH_2O 1 in 10^5 .	With NaNO_2 10 in 10^5 .	With CH_2O 1 in 10^5 and NaNO_2 10 in 10^5 .
May 4, 1912 ...	1.8	1.8	1.8	1.8
May 6, 1912 ...	5.2	3.9	3.2	3.2

Date.	Control.	"Mystin" as recommended.	NaNO_2 10 in 100,000.
May 9, 1912 ...	1.9	1.9	1.9
May 10, 1912 ...	4.2	2.3	2.3
May 11, 1912 ...	7.8	3.3	3.3

Potassium nitrate has sometimes been used as a milk preservative, but its use has not become general on account of its inefficiency. We have done some work showing that it has, however, a slight preservative action. Thus a milk containing 10 parts of potassium nitrate per 100,000 in four days developed an acidity of 5.5, the same sample of milk to which 2 in 100,000 had been added showed an acidity of 5.9, whilst the untreated milk in the same time developed an acidity of 6.2.

In conclusion, we would express our indebtedness to Mr. J. F. Liversseege for his valuable criticism and advice.

CITY OF BIRMINGHAM,
ANALYTICAL DEPARTMENT.