MILK INSPECTION.

PETERS-HILTNER.

DATE DUE

METHODS

FOR THE

EXAMINATION OF MILK

FOR

CHEMISTS, PHYSICIANS AND HYGIENISTS,

COMPILED BY

DR. PAUL SOMMERFELD,

ASSISTANT IN CHEMISTRY IN THE EMPEROR AND EMPRESS FREDERICK HOSPITAL FOR CHILDREN OF BERLIN.

WITH A PREFACE

BY

DR. ADOLF BAGINSKY,

PROFESSOR OF MEDICINE AND SPECIALIST IN DISEASES OF CHILDREN, IN THE UNIVERSITY; DIRECTOR OF THE EMPEROR AND EMPRESS FREDERICK HOSPITAL FOR CHILDREN OF BERLIN.

TRANSLATED BY

DR. A. T. PETERS,

INVESTIGATOR OF ANIMAL DISEASES, UNITED STATES EXPERIMENT STATION OF THE UNIVERSITY OF NEBRASKA.

AND

R. S. HILTNER, A. M.,

INSTRUCTOR IN CHEMISTRY, UNIVERSITY OF NEBRASKA.

CHICAGO:

ALEX. EGER,

1901.

TABLE OF CONTENTS.

PREFACE.

The necessity of providing nurses in children's hospitals with a good article of food in a pure, unadulterated and convenient form for their patients, has led The Emperor and Empress Frederick Hospital for Children to pay special attention to the fulfillment of this need. Definite arrangements were made with an owner of a dairy in close proximity for controlling the selection of cattle, method of feeding, care and milking, thereby securing a good clean product.

The prompt fulfillment of these terms was under the control of Dr. Sommerfield, and it is through his efforts in ascertaining the most expedient and efficient methods of control that this little book has been published.

It will certainly be of interest to those who are entrusted with the care of children in similar institutions to learn of this publication, and we hope, also, that it will be of equal service to many others.

ADOLF BAGINSKY.

TRANSLATORS' PREFACE.

The general excellence of this little work justify the translators in presenting it in this form, with the permission of the author, to the English-reading public. The literature on the subject of milk analysis is by no means limited, but there is a noticeable paucity of manuals or guides adapted to the needs of commercial analysts, market inspectors and health officers.

The translators have adhered as closely as possible to the original text. They have taken the liberty to make a few additions in the way of foot-notes. In cases where the methods given do not accord in detail with those commonly practiced in this country at present, references are made to the standard literature on the subject. A short bibliography has been added in which is catalogued some of the recent researches along lines within the scope of this work.

DR. A. T. PETERS.
R. S. HILTNER.

INTRODUCTION.

Milk, which is a secretion of the lacteals of all female mammalia, is especially characterized by the presence of three elements common to it: casein, butter fat and milk sugar. Besides these there have been found a large number of substances of more or less importance in different kinds of milk.

The mineral substances, phosphoric acid and calcium salts are of importance in aiding the digestibility of milk.

In close relation to the above is the ever present citric acid in cow's milk. In normal milk there was found among other substances, alcohol, acetic acid (Bechamp), ammonia (Latschenberger), milk sugar, urinous substances (Bouchardat, Quevenne, Morin, Picard, Lefort), lecithin, cholesterin, hypoxanthin (Tolmatcheff, Schmidt, Muhlheim), flourine (Wilson), sulpho-cyanic acid (Musso), and kreatinin (Weyl).

The milk of different species is distinguished not only by its physical characteristics and distinct quantities of substances present, but also by the fact that they show themselves different toward various chemical reactions. For instance, the casein of human milk is a different substance from the casein of cow's milk (Biedertfi, Camerer, Lehman). The fat of various kinds of milk presents different chemical reactions, whereas the milk sugar in all kinds is the same (Deniges).

Examination of milk for estimating its value, embraces the quantitative determination of the specific gravity, dry matter, albumin, fat, sugar, ash, phosphoric acid in the ash, and dirt, and the detection of adulterations and preservatives.

QUALITATIVE EXAMINATION OF MILK.

The analyst is seldom called upon to decide whether the fluid under examination is really milk. If such a case should arise, the essential constituents of the material, namely, fat, casein, and milk sugar, should be isolated.

I. FATS.

The fat may be extracted by one of the following methods. By noting the appearance and properties of the butter fat, an index may be had to the quality and source of the milk in question. The ether solution of cows' butter has a faint yellow color; that of human colostrum has an orange or ruby-red color (Pfeiffer), while that of goats' milk is colorless (Schaffer). The milky turbidity of the fluid does not disappear by shaking with ether.

The exact composition of the butter fat in question may be obtained by determining the volatile fatty-acids according to Reichert's method, and the insoluble fatty-acids by Hehner's method. The latter is based on the fact that butter has about 87 per cent. of water-insoluble fatty-acids (varying between narrow limits according to the time of year and the kind of food), while other animal and plant fats contain from 92 per cent. to 95 per cent. of these acids. The analysis, which requires about 200 cc. of milk, is carried out as follows:

Of the previously weighed sample of butter, take three or four grams by means of a glass rod and place

it in an evaporating dish 10 to 12 cm. in diameter. The glass rod with the adhering fat is placed in the dish. The butter sample is again weighed, and in this way the exact amount of butter-fat taken may be found. One now adds to the butter-fat 50 c. c. of alcohol and one to two grams of pure potassium hydrate. The whole is gradually warmed on a water bath, when the butter-fat, especially when stirred, quickly dissolves to a clear, yellow fluid, and a strong odor of butyric acid ester becomes noticeable. The heating is continued for about five minutes, and distilled water is then added, drop by drop. If a cloudiness due to undecomposed fat be noticed, continue to warm it until at last the addition of water to the fluid does not produce the least turbidity. The saponification is then completed. If, through careless addition of water, the fat separates out on the surface as oily drops which will not dissolve in the diluted alcohol, it must be evaporated to dryness and then redissolved in alcohol. In such cases it may be better to commence the process again with fresh material. If the diluting with water is carefully done, it is very rare that a separation of oil occurs.

The clear soap solution is put on the water bath to drive off the alcohol and evaporated to a consistency of syrup. The dish is then removed from the bath and the contents dissolved in 100 to 150 c. c. of water. To decompose the soap, add to this clear fluid a slight excess of dilute sulphuric acid or hydrochloric acid. In this way the insoluble fatty-acids are precipitated out as a curdy mass, which to a great extent rises rapidly to the surface. The material is heated for about half an hour, until the acids melt to a clear oil and until the watery fluid beneath becomes perfectly clear.

In the meantime, select a very thick Swedish filter, 10 cm. diameter, and dry it in the water oven. The filter

paper must be of the best quality and so dense that even hot water will only percolate through it drop by drop. Weigh a small beaker, a funnel, and also the funnel plus the filter. In this way one may get the weight of the filter plus the beaker. (The hardened filters made by Schleicher & Schull are especially adapted to this work.)

The weighed filter is pressed tightly into the funnel and thoroughly moistened and half filled with water. Then pour out of the evaporator the watery fluid and the molten fat, and wash the dish and glass rod with boiling water. It is not difficult to transfer all of the oily mass to the filter, so that there need not be a trace remaining on the dish. To be certain, one may wash the dish with ether and afterwards add the material thus recovered to the insoluble acids. As a rule the amount recovered from the dish with ether in this way is very small, usually less than one milligram.

The fatty-acids are now very carefully washed on the filter with boiling water. Never fill the funnel more than two-thirds full. When the filtrate tested with sensitive litmus paper no longer reacts acid (three grams of fat usually require three-fourths of a litre of boiling water), allow the water to drain out and then dip the funnel and contents into a beaker filled with cold water so that the surface of the water is the same within as without. As soon as the contents of the filter becomes solidified, it is removed from the funnel and placed in the weighed beaker and dried to constant weight in a water oven.

Instead of estimating the insoluble acids as just described, E. Reichert estimates the volatile fatty-acids and recommends the following method for the purpose:*

Two and one-half grams of purified water-free fat, previously filtered through cotton, are placed, in a fluid

*Zeitsch. f. Analyt. Chem. XVIII. 68.

condition, in a flask holding about 150 c. c. (An Erlenmeyer flask is the best form.) To this add one gram of solid potassium hydrate and 20 c. c. of 80 per cent alcohol. This mixture is put on the water bath and agitated frequently, until complete saponification is effected, the mixture becoming perfectly clear and soapy in appearance. Then, taking the flask from the bath, the soap is dissolved in 50 c. c. of water and decomposed by adding 20 c. c. of dilute sulphuric acid (one c. c. of pure sulphuric acid to 10 c. c. of water). The contents of the flask are now subjected to distillation. Care must be taken to prevent the bumping of the fluid, by allowing a light current of air to pass through it. It is also recommended to connect the distilling flask with a large bulbed tube, to prevent the splashing out of the sulphuric acid. Since the distillate, especially from poor butter-fats, or by rapid distillation, always carries with it some solid fatty-acids, it should be immediately filtered through a moist filter and collected in a 50 c. c. flask. After 10 to 20 c. c. has distilled off, pour it back into the flask and again distill, until the flask contains exactly 50 c. c. of distillate. If the distillation has been carried on cautiously, the distillate will be perfectly clear. This distillate is finally transferred to a larger flask and titrated with tenth-normal sodium hydrate, using litmus tincture as an indicator. The titration is considered complete when the blue color of the litmus remains permanent for some time. According to Reichart, 2½ grams of butter-fat require 12.15 c. c. to 14 c. c. of tenth-normal sodium hydrate solution. Figures outside of these limits indicate impure butter-fat.

According to investigations of Pizzi, the milk fat of different mammals shows a slight difference with reference to the amount of volatile fatty-acid contained, which gives a means of determining the source of the milk.

This fact has little practical interest, since in most cases the analyst has to deal only with cows' milk.

II. ALBUMINOIDS.

For the examination of milk for the characteristic albuminoids, dilute the sample with an equal volume of water and add hydrochloric acid to a strong acid reaction and boil. In this way a curdy precipitate of casein and fat is quickly obtained. This is filtered off and washed on the filter with water until all the acid is eliminated. Then to remove the fats wash thoroughly with alcohol and finally with ether. Subject this material to the following reactions which characterize casein.

1. It is readily soluble in very dilute alkali and by careful addition of acid it is reprecipitated.

2. By treatment with acids in excess, solution takes place. It is precipitated from the acid solution by rennet.

3. By boiling the acid or alkali solutions there will be no precipitation.

4. Oxalates of the alkali metals dissolve it producing a milky opalescent fluid. By adding solid magnesium sulphate to the solution a precipitate is formed; simple dilution of the solution does not effect this precipitation.

5. Sodium fluoride dissolves casein, especially when heated. By the introduction of carbon dioxide precipitation takes place.

6. If a sample be placed in a porcelain crucible and fused with a mixture of sodium carbonate and potassium nitrate and then cooled and dissolved in dilute nitric acid, the characteristic reactions of phosphoric acid may be obtained. (Magnesium mixture, ammonium molybdate.) (See page 30).

The positive response to the above described reactions shows conclusively the presence of casein.

III. MILK SUGAR.

To identify the characteristic carbohydrate of milk—milk sugar or lactose—it first must be isolated in a pure state. 100 c. c. to 200 c. c. of milk are treated with acetic acid in the same manner as described, or with rennet to precipitate the casein, and filtered through a linen cloth. The filtrate is then heated for ten minutes to coagulate completely the remaining albuminoids and is again filtered and evaporated to crystallization. After a few days colorless glistening crystals (rhombic prisms) will have separated out. The product may be purified by decanting the mother liquor, dissolving in warm water and recrystallizing. The following reactions are characteristic of milk sugar:

1. It reduces an alkaline copper solution (and Fehling's solution), red cuprous oxide, Cu_2O, being precipitated. Its reducing power is, however, different from that of grape sugar (see page 52). To apply this test, one adds a solution of sodium hydrate to an aqueous solution of the sugar and then adds drop by drop a solution of copper sulphate as long as the resulting blue precipitate dissolves by stirring. By heating this deep blue solution, red cuprous oxide is precipitated.

2. Lactose also reduces alkaline solutions of bismuth, silver and mercury.

3. By boiling a solution of milk sugar for about five minutes with a slight excess of lead subacetate and adding ammonia to the boiling solution until a permanent precipitate is obtained, a cherry red solution results. After a short time a similar colored precipitate settles out (Rubner).

4. If a solution of milk sugar be heated for about an hour upon the water bath with equal parts of phenylhydrazine and acetic acid (50 per cent.), lactosazone is

formed which on cooling separates out in golden yellow needles. The same reaction is effected by heating the sugar solution with two parts of phenylhydrazine hydrochloride and three parts of sodium acetate crystals. By recrystallization out of hot water pure lactosazone may be obtained, melting at 200 degrees C. (The osazone of grape sugar, glucosazone, melts at 205 degrees C.)

5. Milk sugar does not undergo alcoholic fermentation directly, but by boiling with dilute sulphuric acid for an hour and neutralizing with calcium carbonate, the resulting solution will ferment upon the addition of yeast.

6. Lactose turns the plane of polarized light to the right, (a) D equals 52.5 degrees. The rotation will be greater when the solution is boiled for half an hour with dilute acid and made up to its former volume.

7. Unlike grape sugar, solutions of milk sugar will not produce cuprous oxide by boiling with copper acetate and acetic acid.

8. The reaction of nitric acid: To 5 grams of the sugar add 20 c. c. of concentrated nitric acid and cautiously heat the mixture. A very vigorous reaction takes place, producing dense red fumes. When the action of the acid has ceased the reaction product is allowed to stand in a cool place for some time. From the solution mucic acid separates as a crystallized mass. The crystals are thoroughly dried, dissolved in ammonia, and the solution then placed on a water bath and evaporated to dryness. The dried substance when heated in a test tube yields pyrrol, the vapor of which will color dark red a pine splinter previously moistened with hydrochloric acid. Grape sugar, when treated in a similar manner, with nitric acid yields oxalic acid but no mucic acid.

Besides the already mentioned difference in the percentage of volatile fatty acids contained in the fat of milk from different animals, one may decide (according to

Pfeiffer) as to the kind of milk, by noting the action of it when heated, and by the appearance of the curd formed. For instance, colostrum coagulates upon boiling in large irregular patches. Old cow milk and old goat milk form firm flakes which quickly become agglomerated, asses' milk and mares' milk yield a flocculent curd, the small soft flakes floating in a turbid milky fluid. Fresh cow milk, goat milk, sheep milk, asses' milk, mares' milk and human milk, a few weeks after delivery, will not coagulate by boiling; the latter not even by addition of dilute (2 per cent.) hydrochloric acid. The rest coagulate by addition of hydrochloric acid: and asses' milk and mares' milk, as mentioned above, form small, tender flakes, while cows', goats' and sheep's milk yield firm flakes suspended in an almost clear fluid.

METHODS OF QUANTITATIVE ANALYSIS.

I. DETERMINATION OF SPECIFIC GRAVITY.

For an absolutely accurate determination of specific gravity, the pyknometer must be used. The pyknometer is graduated for a definite temperature. The determination of specific gravity therefore must be made at that degree. The milk and the instrument are brought to the proper temperature by placing in warm water. Since the cream readily separates from the milk, it is best to mix the sample thoroughly by shaking before each determination.

The pyknometer is accurately tared, filled with milk and weighed. If the capacity of the instrument be 10 cc. and if it weigh, when filled with milk, after deducting the tare, 0.1035 gram, the specific gravity of the milk would be $10 \times 0.1035 = 1.035$.*

For a less accurate determination of the specific gravity one may use a good areometer (lactodensimeter). On account of the rapid rise of the cream on milk, one must read the scale as quickly as possible. An areometer constructed especially for the examination of milk should have a scale that will indicate the specific gravity accurately to the fourth decimal place. The range of the

*The pyknometers most commonly used at present, have a capacity of about 50 c.c.. They are first dried and weighed; then filled with distilled water at a chosen temperature (usually 17.5° C.) and weighed; and finally rinsed and filled with milk at exactly the same temperature, and again weighed.

Weight of milk divided by weight of water equals specific gravity.—Translators.

scale should be from 1.025 to 1.040, with the marks indicating the second decimal points at least 20 to 25 m. m. apart.

In order to obtain the normal specific gravity reading and thus satisfy the market inspector, skimmed milk is not infrequently adulterated with water. In certain cases one may detect this adulteration in the following manner: The milk in question is placed in a narrow, tall cylinder and shaken thoroughly and tested with the areometer, and then allowed to stand quietly for 24 hours for the cream to rise. After carefully skimming off the cream, the milk is tested again. For good cow's milk (whole milk), the first reading should be from 1.028 to 1.033 and the second from 1.035 to 1.0365. For this purpose the cream is allowed to separate from the milk in a vessel provided with a faucet at the bottom, through which one may readily draw off the cream-free milk into another vessel and then test it with the areometer. The exact composition, however, may be determined only by a complete chemical analysis.

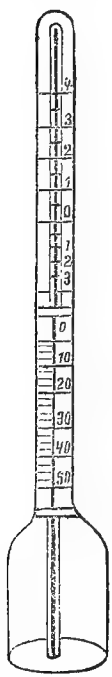

FIG. 1. Upper part of a Lactodensimeter as used by market inspectors.

The "lactodensimeter" designed by Quevenne is an areometer with a scale of 14 degrees to 42 degrees. The degrees of the scale indicate the specific gravity, e. g., 14 represents the specific gravity 1.014. Hence when the temperature is 15 degrees C., the reading shows directly the density of the sample. For temperatures other than 15 degrees C. the proper correction must be made (see tables pages 23 and 24).

TABLE OF CORRECTIONS FOR THE QUEVENNE LACTODENSIMETER.

(WHOLE MILK.)

	8	9	10	11	12	13	14	15	16	17	18	19	20
14	13.2	13.3	13.4	13.5	13.6	13.7	13.8	14.0	14.1	14.2	14.4	14.6	14.8
15	14.2	14.3	14.4	14.5	14.6	14.7	14.8	15.0	15.1	15.2	15.4	15.6	15.8
16	15.2	15.3	15.4	15.5	15.6	15.7	15.8	16.0	16.1	16.3	16.5	16.7	16.9
17	16.2	16.3	16.4	16.5	16.6	16.7	16.8	17.0	17.1	17.3	17.5	17.7	17.9
18	17.2	17.3	17.4	17.5	17.6	17.7	17.8	18.0	18.1	18.3	18.5	18.7	18.9
19	18.2	18.3	18.4	18.5	18.6	18.7	18.8	19.0	19.1	19.3	19.5	19.7	19.9
20	19.1	19.2	19.3	19.4	19.5	19.6	19.8	20.0	20.1	20.3	20.5	20.7	20.9
21	20.1	20.2	20.3	20.4	20.5	20.6	20.8	21.0	21.2	21.4	216	21.8	22.0
22	21.1	21.2	21.3	21.4	21.5	21.6	21.8	22.0	22.2	22.4	22.6	22.8	23.0
23	22.1	22.2	22.3	22.4	22.5	22.6	22.8	23.0	23.2	23.4	23.6	23.8	24.0
24	23.1	23.2	23.3	23.4	23.5	23.6	23.8	24.0	24.2	24.4	24.6	24.8	25.0
25	24.0	24.1	24.2	24.3	24.5	24.6	24.8	25.0	25.2	25.4	25.6	25.8	26.0
26	25.0	25.1	25.2	25.3	25.5	25.6	25.8	26.0	26.2	26.4	26.6	26.9	27.1
27	26.0	26.1	26.2	26.3	26.6	26.6	26.8	27.0	27.2	27.4	27.6	27.9	28.2
28	26.9	27.0	27.1	27.2	27.4	27.6	27.8	28.0	28.2	28.4	28.6	28.9	29.2
29	27.8	27.9	28.1	28.2	28.4	28.6	28.8	29.0	29.2	29.4	29.6	29.9	30.2
30	28.7	28.8	29.0	29.2	29.4	29.6	29.8	30.0	30.2	30.4	30.6	30.9	31.2
31	29.7	29.8	30.0	30.2	30.4	30.6	30.8	31.0	31.2	31.4	31.7	32.0	32.3
32	30.6	30.8	31.0	31.2	31.4	31.6	31.8	32.0	32.2	32.4	32.7	33.0	33.3
33	31.6	31.8	32.0	32.2	32.4	32.6	32.8	33.0	33.2	33.4	33.7	34.0	34.3
34	32.5	32.7	32.9	33.1	33.3	33.5	33.8	34.0	34.2	34.4	34.7	35.0	35.3
35	33.4	33.6	33.8	34.0	34.2	34.4	34.7	35.0	35.2	35.4	35.7	36.0	36.3

TABLE OF CORRECTIONS FOR THE QUEVENNE LACTODENSIMETER.

(SKIMMED MILK.)

18	17.3	17.4	17.5	17.6	17.7	17.8	17.9	18	18.1	18.2	18.4	18.6	18.8
19	18.3	18.4	18.5	18.6	18.7	18.8	18.9	19	19.1	19.2	19.4	19.6	19.8
20	19.3	19.4	19.5	19.6	19.7	19.8	19.9	20	20.1	20.2	20.4	20.6	20.8
21	20,3	20.4	20.5	20.6	20.7	20.8	20.9	21	21.1	21.2	21.4	21.6	21.8
22	21.3	21.4	21.5	21.6	21.7	21.8	21.9	22	22.1	22.2	22.4	22.6	22.8
23	22.3	22.4	22.5	22.6	22.7	22.8	22.9	23	23.1	23.2	23.4	23.6	23.8
24	23.2	23.3	23.4	23.5	23.6	23.7	23.9	24	24.1	24.2	24.4	24.6	24.8
25	24.1	24.2	24.2	24.4	24.5	24.6	24.8	25	25.1	25.2	25.4	25.6	25.8
26	25.1	25.2	25.3	25.4	25.5	25.6	25.8	26	26.1	26.3	26.5	26.7	26.9
27	26.1	26.2	26.3	26.4	26.5	26.6	26.8	27	27.1	27.3	27.5	27.7	27.9
28	27.1	27.2	27.3	27.4	27.5	27.6	27.8	28	28.1	28.3	28.5	28.7	28.9
29	28.1	28.2	28.3	28.4	28.5	28.6	28.8	29	29.1	29.3	29.5	29.7	29.9
30	29.1	29.2	29.3	29.4	29.5	29.6	29.8	30	30.1	30.3	30.5	30.7	30.9
31	30.1	30.2	30.3	30.4	30.5	30.6	30.8	31	31.2	31.4	31.5	31.8	32.0
32	31.1	31.2	31.3	31.4	31.5	31.6	31.8	32	32.2	32.4	32.6	32.8	33.0
33	32.1	32.2	32.3	32.4	32.5	32.6	32.8	33	33.2	33.4	33.6	33.8	34.0
34	33.1	33.2	33.3	33.4	33.5	33.6	33.8	34	34.2	34.4	34.6	34.8	35.0
35	34.0	34.1	34.2	34.3	34.4	34.6	34.8	35	35.2	35.4	35.6	35.8	36.0
36	35.0	35.1	35.2	35.3	35.4	35.6	35.8	36	36.2	36.4	36.6	36.9	37.1
37	36.0	36.1	36.2	36.3	36.4	36.6	36.8	37	37.2	37.4	37.6	37.9	38.2
38	37.0	37.1	37.2	37.3	37.4	37.6	37.8	38	38.2	38.4	38.6	38.9	39.2
39	37.9	38.0	38.2	38.3	38.4	38.6	38.8	39	39.2	39.4	39.6	39.9	40.2
40	38.8	38.9	39.1	39.2	39.4	39.6	39.8	40	40.2	40.4	40.6	40.9	41.2

In this table the figures in the top horizontal line indicates the temperature of the milk and those in the left vertical line indicate the lactodensimeter reading. For instance, if the areometer reading is 30 and the temperature is 16 degrees, the exact specific gravity may be found in the ninth column and the sixth line from the bottom. This figure 30.2 signifies a specific gravity of 1.0302.

Other forms of apparatus described by Bischoff, Recknagel and Soxhlet, do not require the table of corrections. They are provided with a thermometer without the regular thermometric scale. The thermometer reading is simply a correction number which must be either added to or subtracted from the reading on the main scale (see figure 1).

II. THE DETERMINATION OF THE REACTION AND ACIDITY.

The reaction of human milk in its normal state is alkaline; the milk of carniverous animals is acid. Cow's milk should, according to earlier investigators, has an amphoteric reaction. However, in a perfectly fresh condition, that is, at the moment it leaves the udder, cow's milk reacts slightly acid (Vaudin). The reaction does not change if the milk is placed in a hermetically sealed glass tube and heated a long period at a temperature of 100 degrees C. It becomes more strongly acid when allowed to stand in the open air or even with the air excluded. Through the action of bacteria (ferments) milk sugar is converted into lactic acid (commonly called lactic acid fermentation). Ordinarily inactive lactic acid is thus produced, although the formation of active paralactic acid has been observed by Gunther and Thierfelder. Occasionally carbonic acid and ethyl alcohol are formed (Leichmann). The acid production, which is favored by high temperatures, ceases when a fixed amount of lactic

acid has been formed (0.604 per cent., according to Timpe), because the ferment is destroyed when the decomposition reaches a certain degree of concentration wherein the conditions for its growth are removed.

In order to ascertain the age of milk from the strength of the acid reaction, the initial reaction must be known. If the souring has advanced so far that it may be tasted the casein will soon begin to coagulate. The milk is then doubtless old and spoiled. Before this stage is reached, however, one may determine the age of milk with some degree of accuracy, in the following manner: old milk coagulates by treating with carbon dioxide and subsequently heating to boiling. Older milk, without treatment with carbon dioxide coagulates by boiling, while with still older milk coagulation takes place without boiling by simply forcing in carbon dioxide. Quite fresh milk, especially cow milk at the beginning of her lactation period, will sometimes curdle by boiling, but the resulting curd in this case consists not of casein, but of another proteid, viz., globulin. If this point is to be proved, the sample in question is treated with di-sodium phosphate solution till the acidity is almost neutralized, and then boiled. Under these conditions albumin and globulin alone will be precipitated out, if the milk is fresh. On the other hand, if the sample should curdle by boiling without adding di-sodium phosphate it is evident that the milk is old and that fermentation has begun, forming lactic acid. The curd in this case is casein. When alkaline or neutral milk is boiled the curd produced is always albumin.*

*Sembritzki found that by continually removing the scum that forms on boiling milk, he could obtain 1.023 per cent of the milk in this way. Since albumin is not present in milk in such large quantity, it follows that some other body—probably casein—must enter into the composition of this scum or curd. See "Poggendorf's Annalen der Physik und Chemie," 37, page 460.) Translators.

For the quantitative estimation of acidity of milk, a measured quantity of the sample is titrated with dilute sodium hydrate of known strength; 10 c. c. of milk is diluted with 90 c. c. of distilled water and thoroughly shaken. Add one or two drops of a strong alcoholic solution of phenolphthalein for an indicator. From a burette (graduated in one-tenth c. c.), a one-tenth or one-fifth normal solution of sodium hydrate is run in drop by drop with constant stirring, until the bright red color remains permanent. (Phenolphthalein is colored red with alkalies.) The operation is repeated with a second portion of the milk and the mean of the two titrations taken. The results should not differ by more than one-tenth c. c. The acidity is expressed in terms of cubic centimeters of sodium hydrate required. (One should not accept the results of one titration.) According to Pfeiffer, the quantity of acid in different kinds of milk differs according to the time of year, the climate, and other conditions, so that no limits can be fixed. He therefore recommends that every observer find the average acidity coefficient for milk from his locality, by making numerous tests of strictly pure samples, "preferably 'stall samples.'"

The age of milk may be approximately judged by determining the acidity in the following manner: The method is not considered very reliable. 25 c. c. of milk are placed in the flask and a few drops of phenolphthalein added and titrated, as above, with barium hydroxide solution until a faint red tint remains; 1 c.c. of baryta solution should be equivalent to 0.005 grams SO_3, that is, one liter of the solution must contain 10.705 grams of barium hydrate. With fresh milk the red color appears on adding 17 c.c. of the barium hydrate solution. This acidity remains constant at 10 degrees Centigrade for 48 hours, at 15 degrees for 20 hours and at 37 degrees for 5 hours. If milk kept for one hour in a culture oven

shows after that time an increased acidity, it was not fresh and should not be used as food for children.

One may confirm his opinion as to the age of the milk in question by the use of a eudiometer tube. The tube is filled with milk, and the open end placed under mercury to exclude the air. If the milk be old, bubbles of gas will collect in the upper end of the tube. Pure fresh milk after standing 12 hours will develop no perceptible amount of gas. (Schaffer.)

III. DETERMINATION OF TOTAL SOLIDS.

A platinum crucible with the cover (or in the absence of this, a porcelain dish with cover) is accurately weighed and into it is measured five or ten c.c. of the sample which has been thoroughly shaken to render it homogenous. The dish and contents are then placed in a water oven and dried at 100 degrees C. The cover is placed on the dish in such a manner as to allow the escape of steam without permitting dust to enter. After 15 to 20 hours the dish is removed from the oven and allowed to cool over sulphuric acid in a desiccator, and weighed. It is then placed in the drying oven again for half an hour and once more cooled and weighed. If there be no difference between the first and second weighing, the determination may be considered completed. Otherwise the heating must be continued until the weight becomes constant. By deducting the weight of the empty dish from this last weight obtained, the weight of the residue or dry matter is determined. If it be desired to weigh the milk instead of measuring it, the sample is placed in a tared crucible, and weighed quickly. The cover should be kept in place to avoid evaporation. The desiccation of milk by mixing with some porous substance such as gypsum or pumice, or with sand, or by absorbing it with paper, is not to be recommended. Such treatment inter-

feres with the accuracy of the ash determination which usually follows the estimation of total solids.

By prolonged heating the albuminoids of milk acquire a brown color (Renck), and according to Cazeneuve and Haddon and Renck the lactose becomes caramelized, so that the dry residue is always yellowish brown in color. It has been shown that this is not a source of error.

The dry matter is very hygroscopic and therefore the dish must be tightly covered while being weighed.

IV. DETERMINATION OF ASH.

To determine the ash or mineral substances of milk one may use to advantage the residue of dry matter just obtained. This residue is heated to dull redness in the dish loosely covered. After igniting for one to two hours the ash becomes pure white. The crucible and contents are then cooled in the desiccator and weighed. If phosphoric acid in the ash is to be determined, not less than 20 c.c. of milk should be incinerated. The ash is dissolved in very dilute nitric acid and treated with an excess of ammonium molybdate solution. (At least 40 parts of molybdate to one part of phosphoric acid). A yellow precipitate of ammonium phosphomolybdate is obtained. The whole is then allowed to stand for twelve hours at a temperature of about 40 degrees C., and is then filtered through a small filter and the filtrate examined. An addition of ammonium molybdate to the filtrate should cause no further precipitation, even on long standing. The precipitate on the filter is washed with a 10 per cent. solution of ammonium nitrate until the washings cease to react acid and is then dissolved by adding a little warm dilute ammonia. Rinse the filter thoroughly with very dilute ammonia. The filtrate and washings containing the ammonium phosphomolybdate

in solution are collected in the beaker and treated with ammonium chloride (about one-sixth its volume). To this is added while stirring constantly magnesia mixture as long as a precipitate is formed. Finally a slight excess of magnesia mixture is run in and the solution allowed to stand for 12 hours in the cold. The precipitate of ammonium magnesium phosphate is then filtered off and washed with diluted ammonia (1 to 3) until a sample of the filtrate acidified with nitric acid gives no reaction with silver nitrate solution. The filter and contents are then dried at 100 degrees and as much as possible of the precipitate is removed from the paper. The filter is incinerated in a weighed porcelain crucible and the precipitate is then added and the whole ignited at a bright red heat. By this ignition magnesium pyrophosphate is formed, ammonia and water being expelled. If on cooling the residue appears gray, moisten with a few drops of nitric acid, then drive off the acid carefully by heating and again ignite. By this treatment a pure white mass should remain. One part of magnesium pyrophosphate is equivalent to 0.6375 part of phosphoric acid (P_2O_5).

Ammonium molybdate solution is prepared in the following manner: 100 grams of pure molybdic acid are dissolved in 400 grams of ammonia (specific gravity 0.960, equivalent to 10 per cent. NH_3). This solution is poured cautiously into 1500 grams of nitric acid (specific gravity 1.2). The mixture is warmed for about an hour at a temperature not to exceed 50 degrees C. and then kept for two or three days in a moderately warm place. In case molybdic acid precipitates out, the solution should be filtered.

Preparation of magnesia mixture: 55 grams of magnesium chloride and 105 grams of ammonium chloride are dissolved in water, 350 c.c. of ammonia (24 per cent., specific gravity 0.91) added and the solution diluted to

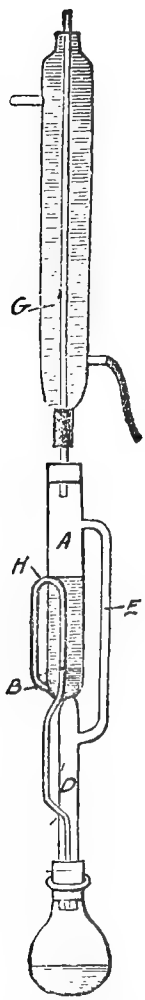

FIG. 2.
The Soxhlet extraction apparatus.

one liter. The solution is kept in a tightly stoppered bottle for a few days and is then filtered.

V. DETERMINATION OF THE PER CENT OF FATS.

For the determination of fats a large number of methods have been proposed, but after a careful examination of the same there are only a few that can be recommended. Of these the method of Liebermann-Weiss is especially commendable for its simplicity of detail. The Soxhlet aerometric method, while it is only applicable to cow's milk, is very satisfactory, and may be carried out without difficulty and with little expense of time. The apparatus, however, is not very cheap.

A. SOXHLET'S GRAVIMETRIC METHOD OF ANALYSIS. (Applicable to all kinds of milk.)

Ten c. c. of the sample are placed in a small porcelain dish containing enough pure sea sand to absorb the milk. The dish and sand should have been previously heated to redness. The dish is placed on the water bath, and the contents stirred and evaporated until the sand appears to be dry. This will be accomplished in about half an hour. The object in keeping the contents well stirred is to prevent the agglomerated sand from adhering to the walls of the dish. The contents of the dish are carefully trans-

ferred, aided by a folded piece of paper, to the extractor. The dish as well as the glass rod and the paper used are finally rinsed with ether into the extraction apparatus. The Soxhlet extraction apparatus is composed of the following parts: see figure 2. The large tube (a) is sealed across at the bottom and is intended to hold the material to be extracted. Into the lower end a narrow tube (b) is sealed and bent upward parallel to tube (a). At the point opposite about the middle of (a), this small tube is curved downward and the end sealed into the stem (d) of the extractor. The tube (e) connects the stem (d) with the upper part of the extractor (a). In the lower end of a tube (a) is placed in a tuft of fat-free cotton (about 2 cm. high). Upon this the substance to be analyzed is placed and the apparatus is connected by means of a tightly fitting cork with a wide necked flask of about 150 c.c. capacity in which is contained about 75 or 80 c. c. of ether. The upper end of the extractor is connected with a Liebig condenser (g). The flask is now warmed on the water bath. The ether vapor passes through the tubes (d) and (e) into the condenser (g). The condensed ether drops back into the tube (a) containing the substance to be extracted. Because of the connection of the tube (b) with (a), the ether is always equally high in both tubes. When the ether reaches the highest point at (h) the tube (b) acts as a siphon and draws the ether, containing the fat in solution, into the flask below. The ether again vaporizes and fills the extractor and siphons off. Thus the process may be continued indefinitely.

The temperature of the water bath is so regulated that within one hour the tube (a) is filled and emptied about twelve times. At the end of this time the extraction may be considered completed. The temperature of the water bath should not be too high, because, if the ether boils

too rapidly the vapor will not be entirely condensed, and a considerable part of it will thus be lost. The quantity of ether used must be sufficient to fill the tube (a) to the top of the siphon tube (b) at least one and a half times. The different parts of the apparatus should be connected by tightly fitting corks or by ground glass joints.

The flask containing the fat in ether solution is placed on the water bath and the ether distilled off. It is then placed in drying oven and dried at 105 degrees C. to constant weight. The weight of the empty flask having been previously ascertained, the increase in weight will indicate the weight of fat in the sample. From this data the per cent of fats may be readily calculated.

Instead of evaporating with sand on the water bath—a process which takes more or less time—Fernandez-Krug and Hampe recommend the mixing of five or ten c.c. of milk with 7.5 to 15 grams of powdered kaolin and 5 to 10 grams of water-fee sodium sulphate. In this way a perfectly dry mass is obtained which may be extracted at once with ether.

Pfeiffer suggests precipitating out the albuminoids and fat of the milk and filtering through a folded filter. The residue is washed a few times with water, dried at 100 degrees and placed with the filter in a Soxhlet extraction apparatus. To precipitate the fats and albuminoids trichloracetic acid or copper hydrate may be used.

B. THE EXTRACTION OF FATS BY MEANS OF PETROLEUM ETHER. (The Lieberman-Weiss Method.)

Liebermann and Szekely and Weiss propose petroleum ether as a solvent in determining the per cent of fat. The method is applicable to cows' and human milk and may be carried out rapidly without the use of a great deal of apparatus. A glass cylinder about 15 cm.

high and about 4 cm. in diameter is chosen and provided with a tightly fitting cork or a glass stopper; 50 c. c. of the milk are placed in the cylinder and treated with 5 c. c. of a potassium or sodium hydrate solution and mixed by shaking. To this is added 50 c. c. of petroleum ether of low boiling point, and 50 c. c. of alcohol (96 per cent). This mixture is shaken two or three times for three minutes and then allowed to stand for half an hour. The upper one of the three noticeable layers consists of petroleum ether with the fat in solution. With the aid of a graduated pipette an aliquot proportion of this solution is drawn off and transferred to a small weighed flask. The petroleum ether is evaporated and the residue of fat is dried at 100 degrees and weighed. By a simple calculation the percentage of fat in the sample is easily determined. For instance, if 25 c. c. of the petroleum ether solution be drawn off and evaporated and one gram of fat be thus obtained, 100 parts of milk will then contain $1 \times 2 \times 2 = 4$ parts of fat, or 4 per cent.

This method has the advantage that the analyst may carry on several control tests with the same original milk sample. It is especially valuable where a very large number of fat estimations are to be made. The results accord very well with the Soxhlet areometric and gravimetric methods. A simple process, a modification of the above, is described by Hoppe and Seyler. It is said to yield good results. Instead of petroleum ether, the authors employ ordinary ethyl ether. Liebermann has criticized this method on the ground that ethyl ether not only dissolves fats but other constituents of milk. A series of experiments made by the author shows that there is scarcely an appreciable difference in action between the two reagents. It may be said in favor of the cheaper fluid, petroleum ether, that it seems to be more

readily removed than the ethyl ether. In any case the gravimetric methods just described are by far the simplest and most quickly carried out.

C. SOXHLET'S AEROMETRIC METHOD. (Applicable only to cow's milk.)

The method depends upon the principle of estimating the specific gravity of the ether solution of the fats. A measured quantity of milk is treated with potassium hydrate solution and ether. By shaking, the fat is taken up by the ether, and the specific gravity of the resulting solution determined in a special apparatus. For this process the following reagents and equipment are necessary:

1. Potassium hydrate solution, specific gravity 1.27 (400 grams of solid caustic potash are dissolved in water and after cooling, the solution is made up to one liter).

2. Ethyl ether, purified by shaking with one-tenth to two-tenths volume of water, and pouring off the clear liquid.

3. Ether, commercial ethyl ether. (Need not be purified.)

4. A vessel of about four liters capacity to be filled with water at 17 to 18 degrees C.

5. Three pipettes; one 200 c. c., one 60 c. c., and one 10 c. c. capacity.

6. Several milk flasks of one-half liter capacity.

7. Two accurate areometers with thermometers: one for skimmed milk, and one for whole milk. These spindles are to be used in a specially constructed cylinder which is kept surrounded with cold water.

8. A rubber hand bellows.

Details of the Method: The milk is brought to a temperature of 18 degrees by holding the bottle in water of the proper temperature. The sample

is well shaken and 200 c. c. of it transferred to a milk flask. It is then treated with 10 c. c. of the potassium hydrate solution and mixed by shaking. To this 60 c. c. of ether, previously saturated with water, are added and the flask then quickly stoppered. The flask is allowed to stand in a water bath at 18 degrees for 15 minutes, and during this time it is shaken every half minute (three or four vertical tosses each time). In this amount of time the ether solution of the fats usually separates out clear, but in case of milk very rich in fat a longer time is required.

The method of procedure may be best explained by reference to the illustration (Fig. 3).

The glass cooler (A) is filled with water at 18 degrees C. The milk flask (B) which contains the ether-fat solution is provided with a two-hole stopper. Into one of the holes is fitted the bent glass tube (C), allowing it to protrude just through the cork. The other end of the tube is connected with the hand bellows (D). Through the other hole of the cork is put a longer glass tube (E) also bent to a right angle. The tube should extend in the bottle almost to the bottom of the layer of ether solution. The end of the tube outside is connected with the stem of the areometer cylinder (F) by a rubber tube. The pinchcock (H) serves to hold the ether solution in (F).

The Operation: By removing the stopper (L) from the cylinder and opening the stopcock (H), the fat solution is forced into the cylinder (F) by pressure from the hand bellows. The cylinder is filled until the areometer (G) floats freely, when the stopcock is quickly closed. By adjusting the screw in the foot of the support the apparatus is brought to a vertical position. The areometer and thermometer readings are then taken and by referring these to the table following, the per cent of

TABLE SHOWING THE PERCENTAGE BY WEIGHTS OF FATS IN WHOLE MILK AS DETERMINED FROM THE OTHER SOLUTION AT 17.5° C.

(SOXHLET'S TABLE.)

Spec. Grav.	Fat Per Cent	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.
43.0	2.07	47.7	2.61	52.3	3.16	56.9	3.74	61.5	4.39
43.1	2.08	47.8	2.62	52.4	3.17	57.0	3.75	61.6	4.40
43.2	2.09	47.9	2.63	52.5	3.18	57.1	3.76	61.7	4.42
43.3	2.10	48.0	2.64	52.6	3.20	57.2	3.78	61.8	4.44
43.4	2.11	48.1	2.66	52.7	3.21	57.3	3.80	61.9	4.46
43.5	2.12	48.2	2.67	52.8	3.22	57.4	3.81	62.0	4.47
43.6	2.13	48.3	2.68	52.9	3.23	57.5	3.82	62.1	4.48
43.7	2.14	48.4	2.70	53.0	3.25	57.6	3.84	62.2	4.50
43.8	2.16	48.5	2.71	53.1	3.26	57.7	3.85	62.3	4.52
43.9	2.17	48.6	2.72	53.2	3.27	57.8	3.87	62.4	4.53
44.0	2.18	48.7	2.73	53.3	3.28	57.9	3.88	62.5	4.55
44.1	2.19	48.8	2.74	53.4	3.29	58.0	3.90	62.6	4.56
44.2	2.20	48.9	2.75	53.5	3.30	58.1	3.91	62.7	4.58
44.3	2.22	49.0	2.76	53.6	3.31	58.2	3.92	62.8	4.59
44.4	2.23	49.1	2.77	53.7	3.33	58.3	3.93	62.9	4.61
44.5	2.24	49.2	2.78	53.8	3.34	58.4	3.95	63.0	4.63
44.6	2.25	49.3	2.79	53.9	3.35	58.5	3.96	63.1	4.64
44.7	2.26	49.4	2.80	54.0	3.37	58.6	3.98	63.2	4.66
44.8	2.27	49.5	2.81	54.1	3.38	58.7	3.99	63.3	4.67
44.9	2.28	49.6	2.83	54.2	3.39	58.8	4.01	63.4	4.69
45.0	2.30	49.7	2.84	54.3	3.40	58.9	4.02	63.5	4.70
45.1	2.31	49.8	2.86	54.4	3.41	59.0	4.03	63.6	4.71
45.2	2.32	49.9	2.87	54.5	3.43	59.1	4.04	63.7	4.73
45.3	2.33	50.0	2.88	54.6	3.45	59.2	4.06	63.8	4.75
45.4	2.34	50.1	2.90	54.7	3.46	59.3	4.07	63.9	4.77
45.5	2.35	50.2	2.91	54.8	3.47	59.4	4.09	64.0	4.79
45.6	2.36	50.3	2.92	54.9	3.48	59.5	4.11	64.1	4.80
45.7	2.37	50.4	2.93	55.0	3.49	59.6	4.12	64.2	4.82
45.8	2.38	50.5	2.94	55.1	3.51	59.7	4.14	64.3	4.84
45.9	2.39	50.6	2.96	55.2	3.52	59.8	4.15	64.4	4.85
46.0	2.40	50.7	2.97	55.3	3.53	59.9	4.16	64.5	4.87
46.1	2.42	50.8	2.98	55.4	3.55	60.0	4.18	64.6	4.88
46.2	2.43	50.9	2.99	55.5	3.56	60.1	4.19	64.7	4.90
46.3	2.44	51.0	3.00	55.6	3.57	60.2	4.20	64.8	4.92
46.4	2.45	51.1	3.01	55.7	3 59	60.3	4.21	64.9	4.93
46.5	2.46	51.2	3.03	55.8	3.60	60.4	4.23	65.0	4.95
46.6	2.47	51.3	3.04	55.9	3.61	60.5	4.24	65.1	4.97
46.7	2.49	51.4	3.05	56.0	3.63	60.6	4.26	65.2	4.98
46.8	2.50	51.5	3.06	56.1	3.64	60.7	4.27	65.3	5.00
46.9	2.51	51.6	3.08	56.2	3,65	60.8	4.29	65.4	5.02
47.0	2.52	51.7	3.09	56.3	3.67	60.9	4.30	65.5	5.04
47.1	2.54	51.8	3.10	56.4	3.68	61.0	4.32	65.6	5.05
47.2	2.55	51.9	3.11	56.5	3.69	61.1	4.33	65.7	5.07
47.3	2.56	52.0	3.12	56.6	3,71	61.2	4.35	65.8	5.09
47.4	2.57	52.1	3.14	56.7	3.72	61.3	4.36	65.9	5.11
47.5	2.58	52.2	3.15	56.8	3.73	61.4	4.37	66.0	5.12
47.6	2.60								

fats is determined. At a temperature of 17½ degrees C. no correction is necessary. Otherwise, for each one-tenth degree above this point add to the areometer reading one degree, or for each one-tenth below, deduct one degree.

For very poor milk (low in fats), at times, a clear ether

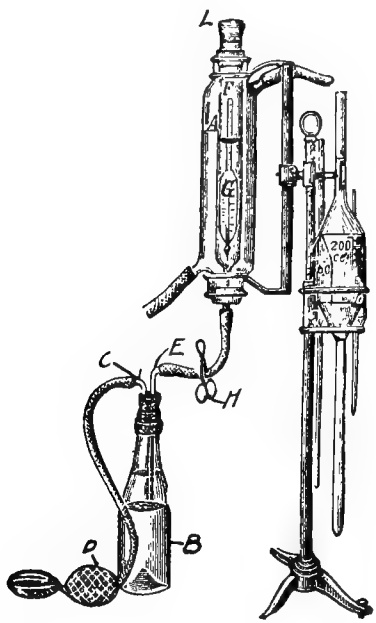

FIG. 3.

fat solution will not separate out, but forms a gelatinous mass. In such cases Soxhlet suggests the addition of from 20 to 25 drops of a soap solution before mixing with ether. The soap solution for this purpose is prepared by heating on a water bath 15 grams of stearic acid[1] with about 25 c. c. of alcohol (96 per cent)

[1]An ordinary stearin candle will answer the purpose.

and 10 c. c. of the above mentioned potassium hydrate solution. Continue heating until a clear yellow solution is obtained, and then dilute to 100 c. c.

D. METHODS WHICH GIVE ONLY APPROXIMATELY ACCURATE RESULTS.

A large number of methods and apparatus have been proposed for the rapid estimation of fats, but most of them are accurate only to a moderate degree. To this class belong the "cremometer," the "lactoscope," the "lactocrite," and others. The first of these depends upon the principle of measuring the layer of cream that separates out; the others are optical methods. The cream measure (cremometer) would give reliable results, if in each case, equal volumes of cream of the same fat content would separate out when samples of milk of uniform fat percentage are allowed to stand in proper vessels under similar conditions. But this is not the case, and moreover, the temperature, the kind of vessel, and the condition of the milk, all have an influence upon the amount of cream obtainable. Thus for example, milk diluted with water, furnishes proportionately more cream than the same milk undiluted, because the fat globules in the watered milk can rise to the surface more easily than in the pure milk, which is specifically heavier.

For similar reasons the optical methods for the more exact determinations should be rejected. The transparency of milk bears a certain relation to the number of fat globules contained in it. That is to say, the larger the number of fat globules, the greater will be the opacity of the milk column. If now the number of fat globules were proportional to the fat content, this method, based upon the transparency of milk, could not be criticized. But this is not the case.

TABLE SHOWING THE PERCENTAGE BY WEIGHT OF FATS IN SKIMMED MILK, AS DETERMINED FROM THE SPECIFIC GRAVITY OF THE ETHER SOLUTION AT 17.5° C.

(SOXHLET'S TABLE.)

Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.	Spec. Grav.	Fat Per Ct.
21.1	0.00	25.5	0.41	29.9	0.82	34.3	1.22	38.7	1.64
21.2	0.01	25.6	0.42	30.0	0.83	34.4	1.23	38.8	1.65
21.3	0.02	25.7	0.43	30.1	0.84	34.5	1.24	38.9	1.66
21.4	0.03	25.8	0.44	30.2	0.85	34.6	1.24	39.0	1.67
21.5	0.04	25.9	0.45	30.3	0.86	34.7	1.25	39.1	1.68
21.6	0.05	26.0	0.46	30.4	0.87	34.8	1.26	39.2	1.69
21.7	0.06	26.1	0.47	30.5	0.88	34.9	1.27	39.3	1.70
21.8	0.07	26 2	0.48	30.6	0.88	35.0	1.28	39.4	1.71
21.9	0.08	26.3	0.49	30.7	0.89	35.1	1.29	39.5	1.72
22.0	0.09	26.4	0.50	30.8	0.90	35.2	1.30	39.6	1.73
22.1	0.10	26.5	0.50	30.9	0.91	35.3	1.31	39.7	1.74
22.2	0.11	26.6	0.51	31.0	0.92	35.4	1.32	39.8	1.75
22.3	0.12	26.7	0.52	31.1	0.93	35.5	1.33	39.9	1.76
22 4	0.13	26.8	0.53	31.2	0.94	35.6	1 33	40.0	1.77
22.5	0.14	26.9	0.54	31.3	0.95	35.7	1.34	40.1	1.78
22.6	0.15	27.0	0.55	31.4	0.95	35.8	1.35	40.2	1.79
22.7	0 16	27.1	0.56	31.5	0.96	35.9	1.36	40.3	1.80
22.8	0.17	27.2	0.57	31.6	0.97	36.0	1.37	40.4	1.81
22.9	0.18	27·3	0.58	31.7	0.98	36.1	1.38	40.5	1.82
23.0	0.19	27.4	0.59	31.8	0.99	36.2	1.39	40.6	1.83
23.1	0.20	27.5	0.60	31.9	1.00	36.3	1.40	40.7	1.84
23.2	0.21	27.6	0.60	32.0	1.01	36.4	1.41	40.8	1.85
23.3	0.22	27.7	0.61	32.1	1.02	36.5	1.42	40.9	1.86
23.4	0.23	27.8	0.62	32.2	1.03	36.6	1.43	41.0	1.87
23.5	0.24	27.9	0.63	32.3	1.04	36.7	1.44	41.1	1.88
23.6	0.25	28.0	0.64	32.4	1.05	36.8	1.45	41.2	1.89
23.7	0.25	28.1	0.65	32.5	1.05	36.9	1.46	41.3	1.90
23.8	0.26	28.2	0.66	32.6	1.06	37.0	1.47	41.4	1.91
23.9	0.27	28.3	0.67	32.7	1.07	37.1	1.48	41.5	1.92
24.0	0.28	28.4	0.68	32.8	1.08	37.2	1.49	41.6	1.93
24.1	0.29	28.5	0.69	32.9	1.09	37 3	1.50	41.7	1.94
24.2	0.30	28.6	0.70	33.0	1.10	37.4	1.51	41.8	1.95
24.3	0.30	28.7	0.71	33.1	1.11	37.5	1 52	41.9	1.96
24.4	0-31	28.8	0.72	33.2	1.12	37.6	1.53	42.0	1.97
24.5	0.32	28.9	0.73	33.3	1.13	37.7	1.54	42.1	1.98
24.6	0.33	29.0	0.74	33.4	1.14	37.8	1.55	42.2	1.99
24.7	0.34	29.1	0.75	33.5	1.15	37.9	1.56	42.3	2.00
24.8	0.35	29 2	0.76	33.6	1.15	38.0	1.57	42.4	2.01
24.9	0.36	29.3	0.77	33.7	1.16	38.1	1.58	42.5	2.02
25.0	0.37	29.4	0.78	33 8	1.17	38 2	1.59	42.6	2.03
25.1	0.38	29.5	0.79	33.9	1.18	38.3	1.60	42.7	2.04
25.2	0.39	29.6	0.80	34.0	1.19	38.4	1.61	42.8	2.05
25.3	0.40	29.7	0.80	34 1	1 20	38.5	1.62	42.9	2.06
25.4	0.40	29.8	0.81	34.2	1.21	38.6	1.63	43.0	2.07

The optical methods have, moreover, the disadvantage that they are dependent upon the individual perception of each observer. Naturally each eye is not equally sensitive in detecting differences of luminosity. Outside conditions under which the test is carried on, also exert an influence on the result. Even for one and the same eye the sensitiveness of the test is not the same in bright sunlight as in cloudy weather, nor in artificial light, as in daylight, etc. Although these methods are not practicable for very accurate work, they are useful to confirm the doubtful results obtained by more exact determinations. They are valuable to the physician, the pharmacist, and especially to the market inspectors, to detect occasional adulteration. The processes have the further advantage that the observer need not be especially skilled. For a close decision of the quality of a sample, however, they are not suited, for the reason just stated.

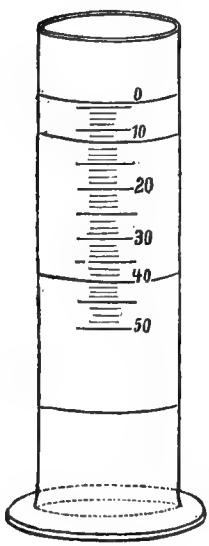

FIG. 4.
The Chevalier Cremometer.

The cremometer of Chevalier (Fig. 4) consists of a glass cylinder 20 cm. high and 4 cm. in diameter, with a capacity of 160 c. c.

It is provided with a hundred-point scale, the zero mark of which is placed 5 cm. below the top rim. The graduations usually are made only from zero degrees to 50 degrees. For the test, the apparatus is filled to the zero mark with milk and allowed to stand for four hours in a room where the temperature is moderate and uni-

form. The cream layer is then measured on the scale. For whole milk the average is 10 to 15 degrees and for half-skimmed milk, 5 to 8 degrees. In order to decide from the reading whether the milk has been skimmed or watered, one must determine the density of the sample thus freed from cream by the method given in the chapter on specific gravity. (See page 22.)

FIG. 5.
The Feser Lactoscope.

Of the many forms of apparatus adapted to the optical methods, one of the best is the lactoscope devised by Feser (Fig. 5). A wide glass tube contains in the constricted lower end a milk-glass cylinder, the wall of which is 4.75 mm. from the surrounding tube. At regular intervals on this cylinder, several equally heavy black bands are glazed. The surrounding tube is provided with a double scale; the left graduated from 0 to 200 and the right from 0 to 10. For the determination the tube is filled with the milk sample to the zero mark. Water is then added until after shaking the black lines of the milk glass cylinder just become visible. The percentage of fats is indicated directly by the right hand scale at the surface of the liquid.

By the Marchand-Tollens' Lactobutyrometer (Fig. 6) results are obtained more easily but are not so accurate.

Ten c. c. of milk are placed in the narrow glass tube,

FIG. 6. The Marchand-Tolleus Lactobutyrometer.

acidified with acetic acid and shaken with 10 c. c. of ether. Then adding 10 c. c. of alcohol (96 per cent), the tube is placed in a water bath at 40 degrees and allowed to remain for a short time. The fats dissolve in the ether and collect on the surface. On the graduated scale, the length of the layer of fat solution is read off, and from this figure the percentage of fats in the sample is determined from the tables which accompany the apparatus. In large dairies and creameries a similar method for fat estimations is used almost exclusively. A measured quantity of the milk is treated with a special acid solution, acetic acid or sulphuric acid (for Laval's lactocrite) in a graduated tube, which is placed in a centrifugal machine and revolved at the rate of six thousand to seven thousand turns per minute. By this means the fat separates out as a clear compact layer. According to Laval, Gerber, Demichel, Rose-Gottlieb, and others, these processes, making use of centrifugal force, give results that are very useful in commercial practice. They are especially well adapted to work in large creameries because a large number of tests may be made in a short time.*

*The Babcock Method.—One of the most expeditious methods for ascertaining the percentage of fats in milk is the so-called Babcock test. It is a simplified modification of the lactocrite method described above. The results obtained by the process are very satisfactory, and usually accord well with the results of ether extractions.

In brief the method consists in measuring out, by means of a special pipette, 17.6 c. c. of the milk sample and transferring to a graduated Babcock flask. To this is added 17.5 c. c. of strong sulphuric acid (sp. gr. 1.81 — 1.83). The milk and acid are thoroughly mixed by gently rotating the flask. By this treatment the casein dissolves completely forming a dark brown fluid, and the

IV. ESTIMATION OF ALBUMINOID SUBSTANCES.

A. DETERMINATION OF TOTAL NITROGEN. (According to Kjeldahl.)

Ten c. c. of milk are placed in a round bottom flask c hard refractory glass, and treated with 15 c. c. of pur conc. sulphuric acid. The acid should be added grad ually and mixed thoroughly by shaking. To this is adde one c. c. of metallic mercury or 0.5 gram of mercuri oxide. The flask is supported in an inclined position o a wire gauze, and gently heated, at first with a sma flame. Gradually the heat is increased until the content of the flask begin to boil. This temperature is then main

---

calcium salts precipitate out as insoluble sulphate. The heat c the reaction with the acid is sufficient to melt the fats, and t keep them in a molten condition during the next step of th process. While still hot the flasks are placed in a centrifuga machine and whirled for about five minutes. The speed of th centrifugal should be maintained at 1,000 to 1,200 revolution per minute. After rotating for five minutes, boiling water i added till the column of fats comes entirely within the range c graduations on the neck of the flask. The flasks are then quickl returned to the centrifugal and revolved again for one or tw minutes. In this way the molten fats are collected in a clearl defined column in the graduated stem of the flask. By subtrac ing the number on the scale at the bottom of the column fro the number at the top, the percentage of butter fats is show directly. The extreme points of the meniscus at the top and a the bottom are to be considered as the terminals of the colum The reading should be taken as quickly as possible and befo the fats solidify.

Experience has shown that in this process, the strength of th sulphuric acid used must be approximately as stated. If the ac be too strong it will carbonize the lactose and particles of carbo will rise to the surface of the liquid. On the contrary, if the ac be too dilute, the casein will not be entirely dissolved and part o the unchanged curd will appear on the surface. In either case th line of demarkation of the fat column will not be sharply d fined.

For determining the per cent of fats in skimmed milk and cream, special forms of flasks are used for each. In the case skimmed milk twice the usual quantity of sample is taken, an the reading obtained is divided by two.

For a more complete description of this method see Wiley "Principles and Practice of Agricultural Analysis," Vol. II page 499.—Translators.

tained for an hour and a half. In this way a clear yellowish or colorless solution is obtained which contains all of the nitrogen of the milk in the form of ammonium sulphate. The flask used in this process should be long-necked (about 15 cm.) and should have a capacity of 150 c. c. to 200 c. c. Care should be taken in digesting the sample with sulphuric acid. Sudden heating is liable to cause bumping and spurting and consequent loss of material. Heating on a sand bath is not to be recommended, because the flask will more easily check or crack and because the temperature is difficult to regulate. Instead of mercury, 0.5 gram of anhydrous copper sulphate may be used although, according to Munk, it is necessary in such cases to digest with sulphuric acid for 20 hours, in order to completely convert the nitrogenous matter into ammonium sulphate.* When mercury is used the transformation may be effected in one to one and a half hours. Many analysts recommend heating the substance with sulphuric acid and sulphuric anhydride, or with sulphuric acid and phosphoric anhydride, or with sulphuric acid and potassium bichromate. Numerous experiments have shown, however, that for the determination of nitrogen in milk, pure sulphuric acid is entirely satisfactory.

When the digestion with acid is complete the contents of the flask are cooled, and transferred to a liter distillation flask. The small flask is rinsed several times with distilled water in order to remove every trace of sulphuric acid. To prevent bumping while distilling add a few fragments of pumice stone. Then add 10 c. c. of a 50 per cent solution of potassium sulphide to decompose mercuro-amides (see note below), and finally enough pure sodium hydrate solution to produce a pronounced alkaline reaction.

*If copper sulphate be used, the addition of potassium sulphate later is unnecessary.

A piece of litmus paper may be thrown in for an indicator. When the strong alkali is being added the flask should be immersed in cold water to prevent the solution from becoming hot and consequent loss of ammonia. The distilling flask is now connected with a Liebig condenser and a 150 c. c. to 200 c. c. distilled off. The distillate which contains all of the ammonia, is collected in a flask containing a measured quantity of standard acid. In order to prevent any of the boiling fluid from being carried over mechanically, a Stutzer-Rietmeyer or Koenig safety tube should be used for connecting with the condenser.

Instead of distilling, the ammonia may be driven out by forcing steam through the solution. For this purpose the flask is fitted with a two-hole stopper. Through one hole is put a short safety connecting tube and through the other, a long tube which reaches nearly to the bottom of the flask. This latter tube is connected with a steam generator.

For collecting the distillate and for absorbing the ammonia, a so-called Pelligot tube or a simpler and cheaper Erlenmeyer flask of 500 c. c. capacity may be used. Into the absorption tube or flask 25 c. c. of a fifth normal solution of sulphuric acid are carefully measured and treated with a few drops of Congo-red, rosolic acid or cochineal solution, for an indicator. Twenty-five c. c. of acid is sufficient for 10 c. c. of milk. The ammonia in the distillate is conducted through a condenser and then into the acid.

Sulphuric acid is most satisfactory as an absorption fluid for the ammonia. It is easily titrated and has the advantage that it does not change in strength by long standing. For an indicator any of the following solutions may be employed: Congo-red, one gram in one liter of water: with ammonia produces a red-brown

color. Cochineal, three grams in 50 c. c. of alcohol and 200 c. c. of water: alkalies, especially ammonia, turn it violet. Rosolic acid, two grams in one liter of 50 per cent alcohol, gives with alkalines and ammonia a cherry-red color. Litmus is not applicable in this analysis. The residual sulphuric acid, not neutralized by the ammonia distillate, is titrated with fifth-normal potassium hydrate. From the number of cubic centimeters of sulpuhuric acid used, that is, the number neutralized by the ammonia, the nitrogen content of the milk sample may be calculated. One c. c. of fifth-normal sulphuric acid is equivalent to 0.0034 grams of ammonia or to 0.0028 grams of nitrogen.

From the total nitrogen found, the amount of albuminoid nitrogen may be calculated by multiplying by 0.94 for cow's milk, or by 0.91 for human milk. Since the albuminoids of the latter (casein, globulin, and albumin) contain 15.76 per cent of nitrogen, the percentage of this material is determined by multiplying the per cent of albuminoid nitrogen by 6.34. For cow's milk the multiple 6.37 is used (Munk).*

In order to make a large number of determinations by this method at the same time, a variety of pieces of apparatus have been devised (Hefter, Holbrung and Morgen, Kreusler, and others). A common form of apparatus for the purpose is described below. The digestion rack for the treatment of the samples with sulphuric acid is made as follows: a circular sheet-iron plate supported on standards serves for the platform. Into this are cut six round holes arranged in a circle. Each hole is covered with a wire gauze which is depressed slightly to furnish a receptacle for the round bottom flasks. In the center of the plate is placed a small iron standard which

*Compare the details of these methods with those of the official methods of the Association of Official Agricultural Chemists. Bulletin No. 46, U. S. Dept. of Agr., Division of Chemistry, page 14 et seq.—Translators.

supports a smaller sheet-iron plate. Around the edge of this plate six semi-circular notches are cut, equally distant from one another. The necks of the flasks fit into these notches and are thus supported in an oblique position. For the heating, six Bunsen burners are fitted at proper intervals to a gas pipe bent in the form of a circle. Each burner is controlled by a stopcock. For the distillations a simple iron stand with sheet iron top is used. Six holes are cut through and covered with wire gauze to support the flasks. Bunsen burners fixed to a main gas pipe supply the heat. The condenser consists of a large rectangular tank, supported on a suitable stand and provided with an inlet and outlet for cold water. Through the tank are put six wide glass condensing tubes, the connections being made with tightly fitting rubber stoppers.

B. METHODS OF PRECIPITATION OF ALBUMINOIDS.

In technical analyses and market control tests, where it is unnecessary to determine the exact distribution of the nitrogen in various combinations, the method of Kjeldahl's for the estimation of total nitrogen has proved most satisfactory and reliable. For the direct determination of albuminoid bodies a large number of processes have been proposed. (Alcohol precipitation; precipitation with tannin, precipitation with acetic acid according to Hoeppe-Seyler, and with copper sulphate and sodium hydrate according to Ritthausen.) J. Munk has recently made a study of these methods and has suggested several modifications which tend to simplify the processes and render them more accurate. Only those methods which have proved to yield satisfactory results and which are applicable to all sorts of milk will be considered here. These methods all depend upon the complete precipita-

tion of the albuminoid bodies, and the determination of the nitrogen in the precipitate by the Kjeldahl process. A determination of the nitrogen in the filtrate gives the so-called nitrogenous extract or non-albuminoids.

In order to precipitate every trace of albuminoids from the milk sample, any one of the following substances may be used: Tannin and sodium chloride in the cold (Sebelien's process), copper hydroxide in a boiling solution (Ritthausen-Stutzer), or trichloracetic acid in the cold (Bondsczinsky).

1. Precipitation with Tannin.

In a 250 c. c. beaker, 10 c. c. of milk are diluted with 90 c. c. of water and treated with about 5 c. c. of a saturated aqueous solution of sodium chloride,* and an excess of tannin or of Almen's solution (5 grams tannin; 5 c. c. acetic acid, 50 per cent; and 200 c. c. alcohol, 30 to 50 per cent). The resulting precipitate settles quite quickly from a clear yellow supernatant fluid, free of albuminoids. The liquid is decanted upon a small Swedish filter and the precipitate washed repeatedly by decantation with distilled water, and finally transferred to the filter. The precipitate should be washed until the filtrate shows no reaction for chlorine. Acidify a few drops of the filtrate with nitric acid and add a drop or two of silver nitrate, when if no turbidity ensues, the washing may be considered complete. To entirely remove the soluble matter by washing requires considerable time because the precipitate quickly fills the pores of the paper, allowing the water to percolate but slowly. This difficulty may be largely overcome by washing the precipitate thoroughly by decantation. The precipitate,

*In all kinds of milk, and especially in human milk, the addition of salt is necessary in order to effect a complete precipitation by the tannic acid.

collected on the filter, is finally transferred to a Kjeldahl flask and digested with sulphuric acid in the usual manner.* From this point on the determination is conducted in the same manner as described for total nitrogen. From the albuminoid nitrogen content thus obtained, the percentage of albuminoids is calculated by the formulas given above (see page 45).

2. *The Ritthausen Method (Modification proposed by Munk).*

The albuminoid bodies are precipitated from a boiling solution by Stutzer's copper hydroxide reagent and the nitrogen estimated in it by the Kjeldahl method.

The copper hydroxide is prepared in the following manner: 100 grams of crystallized chemically pure copper sulphate are dissolved in five liters of water and treated with two and a half grams of glycerine. Dilute sodium hydrate is then added which precipitates the copper as cupric hydroxide. The precipitate is then filtered off and washed with water containing 0.5 per cent glycerine by decantation until the filtrate is free of alkali. The filtrate must be entirely colorless indicating the absence of copper. If it be still blue more sodium hydrate must be added. The filtered mass is then treated with water containing 10 per cent of glycerine and so diluted that it may be readily drawn up into a pipette. The mixture should be kept in a tightly stoppered blue or brown bottle. The proportion of copper hydroxide per cubic centimeter may be determined by drying a measured volume of the material and weighing the residue. Before using this reagent it should be shaken vigorously in order to mix the precipitate uniformly.

*The nitrogen content of Swedish filters is usually small but should be determined in a "blank" and proper deduction made in the calculation.

Previous to the precipitation of the albuminoid substances with Stutzer's reagent the milk must be treated with alum solution to decompose the alkaline phosphates contained. Otherwise in the presence of cupric hydroxide these phosphates suffer decomposition in which copper phosphate and free alkali are formed. This latter will then dissolve the albuminoids and render the estimation inexact. By the addition of alum this objectionable reaction of the alkaline phosphates is prevented owing to the formation of alkaline sulphates and insoluble aluminum phosphates.

For the analysis by this process 10 c. c. of milk are placed in a 250 c. c. beaker and diluted with 100 c. c. water (for human milk 60 c. c. water are used). The mixture is then heated nearly to boiling, 2 c. c. of saturated alum solution are added and when the fluid begins to boil 5 c. c. of Stutzer's reagent are run in and the mixture kept at the boiling point for five minutes. The resulting precipitate which settles quickly is washed with hot water until the filtrate shows no reaction for sulphuric acid (the addition of barium chloride to a few drops of the filtrate acidified with hydrochloric acid should produce no turbidity). The filter and contents are then placed in a Kjeldahl flask and treated in the usual manner.

The reaction of the diluted milk should be noticed before adding the copper reagent. If it be alkaline it should be exactly neutralized by the careful addition of alum since free alkali as mentioned above, dissolves the albuminoids and consequently prevents the precipitation by the copper hydroxide. From the nitrogen content thus determined the percentage of albuminoids may be calculated according to the formula given.

3. *Precipitation with Triohloracetic acid* (*Bondsczinsky*).

Ten c. c. of milk are diluted with 50 c. c. of water and treated with 10 c. c. of a 15 per cent. aqueous solution of trichloracetic acid. This causes a complete precipitation of the albuminoids. The reaction takes place in the cold. The precipitate is allowed to stand a few hours and then washed with dilute trichloracetic acid and finally the nitrogen content of the residue determined by the Kjeldahl method.

VII. DETERMINATION OF LACTOSE.

For the estimation of lactose any one of the following methods may be used. First—Titration with Fehling's solution. Second—Gravimetric Analysis by the Soxhlet-Allihn method. Third—Determination by circular polarization.

A. TITRATION WITH FEHLING'S SOLUTION.

Before titrating with Fehling's solution the milk sample must be freed of albuminoids. For this purpose the filtrate obtained from the precipitation of the albuminoids by the Munk-Ritthausen method may be used (page 48). This filtrate, together with all of the wash water is well mixed and made up to a definite volume (usually 200 c. c.). An aliquot portion of this solution is then titrated with Fehling's solution of known reducing power. This reagent is prepared in the following manner. It consists of two solutions which should be kept separate.

Solution 1. 34.639 grams pure crystallized copper sulphate are dissolved in warm water and the solution after cooling made up to exactly 500 c. c.

Solution 2. 173 grams of pure crystallized Siegnette salts (potassium sodium tartrate) and 50 grams chemically pure sodium hydrate are dissolved separately in distilled water and the cooled solutions then united and made up to 500 c. c.

For use in the analysis equal volumes of solutions one and two are mixed together. Since the mixture of the two solutions suffers decomposition by standing, they should be kept separate until required for use. They should be kept in well stoppered bottles.

The following are the details of the process: 10 c. c. of each of the two reagents are mixed in a small porcelain evaporator, diluted with 50 c. c. of water and heated to boiling. Into the solution a few cubic centimeters of the filtrate containing the sugar are run in and heated for a few moments. It is then allowed to stand till the red cuprous oxide settles out. The color of the supernatant liquid is then noted. If it be still blue a few more c. c. of the sugar solution are run in from the burette and again heated and allowed to settle. This process is continued until the solution becomes colorless. Another sample of the Fehling's solution is then measured out and titrated with the sugar solution as before. The previous determination will serve as a guide so that nearly the required amount of sugar solution may be run in at once. The exact end point may then be determined by careful titration drop by drop. At least three tests of this sort should be made. The first observation is usually discarded.

The exact point of complete discoloration of the fluid is often difficult to detect because of the incomplete separation of the precipitate. The use of side tests of potassium ferrocyanide for the determination of the end reaction, as formerly recommended, is considered unsatisfactory. The solution should not be allowed to stand too long in order to permit the precipitate to settle completely because the cuprous oxide easily oxidizes to cupric oxide by the action of the oxygen of the air and dissolves again in the alkaline solution producing a blue color. If the end reaction be difficult to determine with precision, a small sample of the solution may be filtered

out and the filtrate placed in a small test tube and held over a white paper. In this way any blue coloration may be readily detected. In case the solution be still blue this filtered portion may be returned to the evaporator and titrated further with the sugar solution. If the filtrate shows a yellowish tint it is probable that too much of the sugar-bearing filtrate had been used.

The amount of filtrate required to reduce 20 c. c. of Fehling solution is equivalent to 0.134 gram of lactose. Upon this fact, the calculations are based. For example, if one uses 10 c. c. of milk and separates out the fats and the albuminoids and makes up the filtrate to 200 c. c. and finds that 50 c. c. of it are required to reduce 20 c. c. of Fehling's solution, then the percentage of milk sugar contained in the milk will be calculated by the formula 4 x 0.134 x 10, which is equivalent 5.36 per cent.

Where great accuracy is not required this method of titration is useful because results may be quickly obtained. It is especially well adapted for technical analyses, for analyses of food stuffs, and for clinical tests. In cases where a greater degree of accuracy is demanded gravimetric methods of analysis should be used. For standardizing and controlling the Fehling's solution a solution of chemically pure milk sugar or dextrose is used. In the latter case one-tenth of a gram will reduce 20 c. c. of Fehling's solution.

B. SOXHELT AND ALLIHN'S GRAVIMETRIC METHODS.

In estimating the lactose by these methods also, the albuminoids and fats of the milk must be eliminated. For this purpose the modified Ritthausen method is employed. The filtrate is collected in a 200 c. c. flask and the volume completed with water. By using 10 c. c. of milk and making to 200 c. c. the most favorable concentration is

obtained. For conducting the reduction according to this method of Soxhlet's, 100 c. c. of the filtrate is added to 50 c. c. of Fehling's solution, prepared by the formula given above, and boiled for about ten minutes. At the end of this time the reduction is complete and the cuprous oxide which separates out is filtered off. Owing to the difficulty of filtering out this precipitate completely, several processes of filtration have been devised (Soxhlet, Allihn, Pfeiffer). The filtration may be carried on easily and successfully by using a hardened filter (Schleicher and Schull). The filter is fitted tightly into the funnel and saturated with hot water and the hot supernatant liquid cautiously decanted, care being taken to retain the precipitate in the beaker. This precipitate is washed thoroughly with hot water by decantation. The soluble matter in the paper is completely removed by washing with water and finally the precipitate is transferred to the filter and again washed to remove the last traces of soluble copper. By careful manipulation the precipitated cuprous oxide in the majority of cases will be completely retained by the filter. If, however, any of the precipitate should run through it is best to begin the determination again. It is important in every case to remove all traces of the excess of Fehling's solution from the filter before transferring the precipitate. The washing of the cuprous oxide in the beaker should be continued until the washings show no alkaline reaction or give, when acidulated, no test for copper with potassium ferrocyanide. Soxhlet and Allihn suggest the use of a bulbed tube provided with an asbestos filter for carrying on these filtrations.

The precipitate on the filter is dried at 100 degrees and ignited in a porcelain crucible previously dried and weighed. Continue the ignition until the filter is entirely incinerated. The flame is then removed and the copper oxide covered with a thick layer of powdered sulphur.

The cover, provided with a gas inlet tube (Rose crucible), is then put on and hydrogen gas passed in until all of the air is displaced. Finally ignite for fifteen minutes in an atmosphere of hydrogen. By this means the copper oxide is converted into copper sulphide and is weighed as such. From this weight the known weight of filter ash is deducted and the percentage of lactose calculated from the accompanying table.

Instead of converting the cuprous oxide into copper sulphide it may be reduced to metallic copper by heating in an atmosphere of hydrogen and weighing the resulting copper. Or instead of this the precipitated cuprous oxide may be collected on a filter previously dried and weighed and dried at 100 degrees to constant weight. The increase in weight of the filter will indicate the amount of cuprous oxide. A still more simple process is to ignite the filter and contents in a crucible as described above and moisten with a few drops of conc. nitric acid. The acid is then carefully evaporated off and the copper nitrate which is formed decomposed by ignition. From the weight of black cupric oxide thus obtained the amount of milk sugar may be calculated. For very exact determination the cuprous oxide should always be ignited in an atmosphere of pure hydrogen and weighed either as copper sulphide or metallic copper. For the calculation, find that number in the accompanying table which is nearest to but less than the number for the copper sulphide or metallic copper found. Subtract this from the number found and multiply the remainder by the corresponding factor, as given, and add to the result the number for lactose given in the table. For example assume the weight of metallic copper obtained to be 0.2046 grams. The number in the table nearest to and less than this is 0.2040 grams. The lactose equivalent is 0.150 grams and the corresponding factor is 0.78. Then from

TABLES FOR THE CALCULATION OF LACTOSE.

Copper sulphide found	Factor for 1 mg. of copper sulphide	Metallic copper found	Factor for 1 mg. of metallic copper	Lactose
0.1733	0.60	0.1383	0.76	0.100
0.1816	0.60	0.1449	0.76	0.105
0.1899	0.60	0.1515	0.76	0.110
0.1981	0.60	0.1581	0.76	0.115
0.2064	0.60	0.1648	0.76	0.120
0.2147	0.61	0.1714	0.77	0.125
0.2229	0.61	0.1779	0.77	0.130
0.2308	0.61	0.1844	0.77	0.135
0.3390	0.61	0.1910	0.77	0.140
0.2472	0.61	0.1975	0.77	0.145
0.2553	0.62	0.2040	0.78	0.150
0.5634	0.62	0.2106	0.78	0.155
0.2715	0.62	0.2171	0.78	0.160
0.2795	0.62	0.2235	0.78	0.165
0.2876	0.62	0.2300	0.78	0.170
0.2957	0.62	0.2364	0.78	0.175
0.3037	0.62	0.2428	0.78	0.180
0.3118	0.62	0.2493	0.78	0.185
0.3119	0.62	0.2557	0.78	0.190
0.3280	0.62	0.2621	0.78	0.195
0.3360	0.62	0.2686	0.78	0.200
0.3441	0.62	0.2750	0.78	0.205
0.3522	0.62	0.2815	0.78	0.210
0.3602	0.62	0.2879	0.78	0.215
0.3693	0.62	0.2944	0.78	0.220
0.3764	0.62	0.3008	0.78	0.225
0.3844	0.62	0.3072	0.78	0.230
0.3925	0.62	0.3137	0.78	0.235
0.4005	0.62	0.3201	0.78	0.240
0.4087	0.62	0.3266	0.78	0.245
0.4167	0.62	0.3330	0.78	0.250
0.4248	0.65	0.3391	0.82	0.255
0.4324	0.65	0.3452	0.82	0.260
0.4401	0.65	0.3513	0.82	0.265
0.4477	0.65	0.3575	0.82	0.270
0.4554	0.68	0.3636	0.86	0.275
0.4627	0.68	0.3694	0.86	0.280
0.4700	0.68	0.3752	0.86	0.285
0.4773	0.68	0.3811	0.86	0.290
0.4846	0.68	0.3869	0.86	0.295
0.4919	0.68	0.3921	0.86	0.300

this the amount of copper found represents (0.2046—0.2040)x0.78+0.150=0.0006x0.78+0.150=0.1505 grams of lactose. In case the precipitate is weighed as cuprous oxide (Cu_2O) or a cupric oxide (CuO), the following additional data is required to complete the calculation: One gram of cuprous oxide represents 0.8882 gr. of metallic copper and 1 gram of cupric oxide represents 0.7989 grams of copper.*

C. ESTIMATION BY MEANS OF CIRCULAR POLARIZATION.

In a flask of about 150 c. c. capacity are placed 50 c. c. of milk; 25 c. c. of neutral lead acetate are added, thoroughly mixed by shaking, and heated to boiling. In order to prevent the loss of water by evaporation, the flask is connected by means of a cork to a long glass tube which serves as a condenser. After heating for a short time, the contents of the flask are cooled to room temperature and filtered through a dry filter. The filtrate should be perfectly clear, otherwise it must be filtered again. In some cases it may be necessary to filter several times. With the clear filtrate fill an observation tube 200 mm. long, taking care to force out all the air bubbles and determine the angle of rotation in a suitable polarization apparatus. The specific rotatory power of lactose at 20 degrees is + 52.53 circular degrees, for sodium light. If the angle be (α), and if the tube used be 200 mm. (2 x 100) long, then the number of grammes of lactose per 100 c. c. of milk will be calculated by the formula

$$(\alpha) \cdot \frac{100}{52.53} \cdot \frac{3}{2} \cdot \frac{1}{2} = (\alpha) \cdot 1.4277$$

*Compare these methods with the methods adopted by the Association of Official Agricultural Chemists. See Bulletin No. 46 U. S. Dept. of Agr., Division of Chemistry, page 40-41.—Translators.

The polarization may be made in a Landolt's half shadow instrument. The observation tube should first be filled with distilled water, put in place in the polariscope and a series of readings taken to establish the zero point: that is, the point at which both halves of the field are equally illumined. When this point is fixed exactly, the tube is filled with the filtrate to be examined, and the polariscope reading is made. The field is adjusted until the light on both sides is again of equal intensity. Several readings should be made in order to insure greater accuracy.

VIII. DETERMINATION OF CITRIC ACID.

In the ordinary commercial analyses the estimation of this acid may be omitted, since, from the amount of it present, no conclusions as to the quality of the milk may be drawn. To isolate the acid, a large quantity (about 20 liters) of the milk is used. This is first skimmed and then allowed to coagulate. The serum is clarified by heating with acetic acid, and then filtered. To the filtrate lead acetate solution is added. The resulting precipitate is filtered off and washed and suspended in water and decomposed with hydrogen sulphide. The lead sulphide formed is filtered off and the filtrate evaporated. The residue is cooled, and extracted with ether. The ether solution is then placed in a small weighed dish and evaporated to dryness on a water bath. The residual citric acid is finally dried over concentrated sulphuric acid in a desiccator and weighed. (Vaudin's Method.)

The volumetric method for citric acid, proposed by A. Scheibe, depends upon the precipitation of the acid by means of alcoholic ammonia solution, and the titration of the triammonium citrate with chromic acid. The latter is reduced to chromium sesquioxide and the citric acid

*One litre of cow milk contains 0.9 to 1.0 grams of citric acid in the form of calcium citrate. Henkel, Jahresber, u die Fortsch, der Thierchem., 1888, 94; 1891, 129; Schiebe, ibid 1891, 130.—Translators.

salt oxidized at the same time to carbon dioxide and water.

A solution of ferrous sulphate is used to indicate the end reaction. At the moment when all the citric acid is decomposed, the excess of chromic acid oxidizes the ferrous salt to the ferric condition, the change being recognized by the action of potassium ferricyanide (ferric salts give no precipitate with potassium ferricyanide, while ferrous compounds give a blue precipitate). The process is carried out in the following manner:

"400 c. c. of milk* are mixed with 4 c. c. of sulphuric acid (2½ times normal strength) and heated to boiling; 10 grams of kaolin are mixed with enough water to form a thin paste and added to the above. The mixture is again boiled and then allowed to cool. When cold it is diluted to 500 c. c. and filtered. If the filtrate is not clear it is again treated with kaolin. To 100 c. c. of the filtrate add enough barium hydroxide solution to completely precipitate the sulphuric acid present, and evaporate to a syrup. This residue, which should be stirred to render as homogeneous as possible, is treated with 2.2 c. c. of sulphuric acid (2½ normal) and 20 c. c. of absolute alcohol, and after allowing to stand for a short time is mixed with 50 c. c. of ether. The mixture is then filtered through cotton. In this way the lactose which settles out as a crystalline precipitate, is completely separated. The filtrate is now treated with alcoholic ammonia, the reagent being added as long as any turbidness is produced in the solution. The ether is distilled off, leaving about 20 c. c. of residue. This is treated with 60 c. c. of absolute alcohol and 10 c. c. of alcoholic ammonia. By this means the citric acid separates out as the triammonium salt. On standing a well crystallized

*From Hoppe & Seyler's "Handbuch der Physiologisch—und Pathologisch—Chemischen Analyse," VI. page 471.

precipitate is formed which contains all of the citric acid together with some ammonium chloride and a little organic matter. This latter may be separated by repeating the precipitation.

"The ammonium citrate is dissolved in water and concentrated to about 20 c. c. and titrated with standard potassium bichromate. A solution containing 46.1 grams $K_2Cr_2O_7$ in a liter, is titrated against a solution of ferrous ammonium sulphate, prepared by dissolving 150 gr. of the salt in 700 c. c. water, adding 100 c. c. of concentrated sulphuric acid and completing the volume to one liter; 20 c. c. of this iron solution diluted with 80 c. c. of water requires 7.7 to 7.8 c. c. of bichromate solution for complete oxidation. For the titration of citric acid, the 20 c. c. of this citrate solution are treated with 20-30 c. c. of bichromate solution and 20-25 c. c. of concentrated sulphuric acid and carefully mixed by stirring. After heating for about a quarter of an hour the oxidation to carbonic acid may be considered complete (the solution should not be heated to boiling). The solution is then diluted with 50 c. c. of water and treated with an excess of ferrous ammonium sulphate until the reddish brown color is completely changed to green. With the standard bichromate the solution is titrated back until no more ferrous salts can be detected with potassium ferricyanide. Theoretically 4.61 grams of potassium bichromate are equivalent to 1 gram of citric acid. Numerous titrations have shown, however, that in practice 4.61 grams $K_2Cr_2O_7$ represent 1.02 grams of citric acid. The lactic acid contained in the milk does not affect the results because this acid is not precipitated by alcoholic ammonia."*

*See also "Zeitschift fur Analytische Chemie," 1899, page 718; also "Analyst," 23, page 161; and also "Revue Chim. Analyt. Appl." VI (7) 110.—Translators.

DETECTION OF PRESERVATIVES.

In order to prevent milk from souring and to deceive the customer as to the freshness of it, a number of different preparations are made use of as preservatives. In some cases these preservatives are used for the purpose of preventing the curdling of the milk samples which are to be subjected to analysis, and which for example have to be sent a long distance during hot summer months. To such a procedure no objection could be raised. If, however, milk thus treated be offered for sale as food, vigorous protestations should be made against it from a hygienic standpoint. Such milk should be rejected as food and by all means as food for infants. All assertions and opinions to the contrary are prejudicial and unreliable.

The objection to the use of preservatives is based on the fact that they hinder or prevent coagulation. On this account it is impossible to judge of the age of the milk or to decide whether it is fit for use. Concerning the value of various preparations commonly used as preservatives, it may be said that sodium carbonate and sodium bicarbonate are not especially active as germicides. In fact, many of the pathogenic micro-organisms such as cholera bacilli, develop only in alkaline culture-media. Borax and boric acid act slightly as bacteriacides, while formalin and salicylic acid act strongly. The latter, however, is entirely ineffective against typhus bacilli. Benzoic acid and hydrogen peroxide are effective only when used in large quantities.

In practice the following are quite extensively used: borax and boric acid, sodium carbonate, salicylic acid, benzoic acid, formalin, chromium—, mercury—, and ammonium salts, and sodium fluoride. The last four are used only for analytical work. The first are found most frequently in milk as sold by the unscrupulous purveyor.

For this reason improved methods for detecting these substances are given below.

I. BORAX AND BORIC ACID.

For qualitative tests about 100 c. c. of milk are made alkaline with milk of lime, evaporated to dryness and incinerated. The resulting carbon need not be completely burned. The heating is stopped as soon as intumescence ceases. The ash is dissolved in the least possible amount of hydrochloric acid; filtered off from the carbon; evaporated to dryness; and the excess of HCl completely driven off. The resulting white crystalline residue is treated with a few drops of a tincture of tumeric and very dilute hydrochloric acid and dried on a water bath. The presence of the slightest trace of boric acid gives to the dry residue a beautiful vermillion or cherry red color (Meissel). By the described method it is possible to detect with certainty 0.001 to 0.0005 gram of boric acid in the ash or 0.001 to 0.002 per cent in the milk.

To render the milk alkaline, lime water is preferable to the caustic alkalies because, in fusing, the latter are liable to cover over particles of unburned organic matter and make the conversion to ash difficult. Only very dilute hydrochloric acid must be used in the final evaporation of the crystalline residue in testing for boric acid since the concentrated acid itself gives with tumeric tincture a red color. The coloration produced by boric acid is distinguished from that produced by hydrochloric acid by the fact that it does not disappear by treatment with water in the cold, but only after long boiling. The color caused by hydrochloric acid disappears as soon as it is diluted with water. The acid is easily soluble in alcohol and a trace of it in alcoholic solution colors the flame of the Bunsen burner an intense green. With alcohol, boric

acid ester is formed. If the alcohol be ignited a flame tinged with green is produced.

For the quantitative determination of boric acid Cassal proposes the following process: 100 grams of milk are made alkaline with sodium hydrate, evaporated and incinerated. The ash is washed with water and methyl alcohol into an Erlenmeyer flask fitted with a two hole stopper. Through one of the holes is placed an adapter projecting a short distance into the neck of the flask and connected with a condenser. Through the other hole is placed a separatory funnel filled with methyl alcohol. The contents of the flask are acidified with acetic acid and placed in an oil bath and subjected to distillation. The distillation product is collected in a platinum crucible filled with pure quicklime. This is placed in a large glass beaker which is covered with a glass plate with a hole in the center. Before using, the crucible and contents should be ignited and weighed. Through the perforation in the plate a bent glass tube connected with the free end of the condenser is passed, allowing it to extend down to the crucible. The distillation is repeated about ten times, shaking each time with 5 c. c. of methyl alcohol. The crucible is finally dried and heated strongly with a blast. The increase in weight gives the amount of boric acid in 100 c. c. of milk.

II. SODIUM CARBONATE, SODIUM BICARBONATE.

Ten c. c. of milk are mixed with 10 c. c. of alcohol (96 per cent) and a drop of rosolic acid solution (1:100). Pure unadulterated milk produces a brownish-yellow color, but in the presence of sodium carbonate or sodium bicarbonate a rose color is obtained. For greater precision in doubtful cases the questionable sample should be

compared with known unadulterated milk. Phenolphthalein solution may not be used, as an indicator in place of rosolic acid in this test. By the method given 0.05 per cent of these carbonates may be easily detected (Hilger, E. Schmidt).

III. SALICYLIC ACID.

(a) Acidify 20 c. c. of milk with two or three drops of sulphuric acid and shake with about an equal amount of ether. The greatest possible part of the ether solution is drawn off and evaporated; the residue extracted with 40 per cent alcohol; filtered; and 5 c. c. of the filtrate treated with a few drops of ferric chloride. A violet color shows the presence of salicylic acid. By comparison with a solution of salicylic acid of known strength and colored by treatment with ferric chloride the approximate amount of acid present may be estimated. (Remont.)

(b) 100 c. c. of milk are diluted with 100 c. c. of water at 60 degrees and treated with eight drops of mercuric nitrate, thoroughly shaken, and the resulting precipitate filtered off. The filtrate which will contain all of the salicylic acid originally in the milk sample is shaken with 50 c. c. of ether; the ether solution is drawn off, filtered through a dry filter and allowed to evaporate spontaneously in the air. If the residue resulting be white and crystalline, soluble in alcohol and produces a violet color with a few drops of one per cent ferric chloride solution the presence of salicylic acid is proved conclusively. (Girard.)

IV. BENZOIC ACID.

A large quantity of milk (300-500 c. c.) is made alkaline with calcium or barium hydroxide solution; evaporated to one-fourth its volume; mixed with pure sea sand, pulverized pumice stone or gypsum, to a thick paste and

evaporated to dryness on a water bath. The mass is then finely pulverized, moistened with dilute sulphuric acid and shaken with twice its volume of cold fifty per cent alcohol. The alcohol dissolves out milk sugar, salts, and benzoic acid; only a trace of fats dissolves. The solution is neutralized with barium hydrate and without filtering off the barium sulphate which separates out, evaporated to small volume (10 c. c.) and again acidified with dilute sulphuric acid and extracted three or four times with a small amount of ether. By evaporation of the extracts pure solid benzoic acid remains. (The residue is usually mixed with a trace of fats.)

For a quantitative determination the above residue is dried at 60 degrees or over concentrated sulphuric acid, weighed, and then heated upon a water bath until the benzoic acid sublimes off. In order to identify the benzoic acid subsequently, the sublimate should be collected. This may be accomplished by inverting an evaporator over the other dish. Finally the dish and residue is weighed back and the loss of weight, that is, the difference between the two weighings, indicates the weight of benzoic acid.

The presence of benzoic acid may be confirmed by subjecting the sublimate to the following tests: (1) A small amount is heated gently in a watch glass, over which is inverted another glass of the same size. The acid, if present, sublimes and condenses on the upper watch glass in fine white glistening flakes. (2) A small sample is treated with a few drops of fuming nitric acid, and evaporated to dryness. The dry residue is mixed with sand and heated strongly in a glass ignition tube. In consequence of the formation of nitrobenzol a strong odor of bitter almond oil will be noticed.

V. FORMALIN.

This substance, which has now come into general use as a food preservative, according to some authorities, may be properly used as a preservative for milk. The commercial preparation "Formalin" consists of a 40 per cent solution of formaldehyde in water. The detection of it in milk is a simple matter.* By adding a solution of silver nitrite to the milk a black precipitate is formed when formaldehyde is present. In case of very small amounts of formalin, the nitrite solution produces usually, on standing, only a brownish black stain or coloration, due to the production of finely divided metallic silver. The preservative action of formalin is especially marked. It has been found that milk may be kept 100 hours at 25 degrees C. when formalin is added in the proportion of one part of formaldeyde to 5,000 parts of milk. When added in such small amounts, formalin changes neither the odor nor the taste of milk. Notwithstanding this, its use as a preservative of milk intended for food should be prohibited since it has been shown that even with dilute solutions it retards digestion and also alters the composition of the milk. Upon the albuminoids especially it exerts an influence tending to increase the solubility of

*For the detection of formaldehyde in milk a method has recently been proposed making use of phloroglucin solution. 0.1 gram of phloroglucin is dissolved in 100 c. c. of water. 1-2 c. c. of this solution is treated with a few drops of potassium or sodium hydroxide and added to eight or ten c. c. of the milk to be tested. In the presence of formaldehyde a red coloration is produced. According to Jorrissen one part in 20,000 may be easily detected in this way. This reaction with phloroglucin takes place only with dilute formaldehyde solutions. Vanino found that ten to thirty per cent solutions gave no reaction, or at best only a slight coloration. A three per cent solution produces a deep red color. The strongest color is produced by 0.5 per cent. With solutions containing 0.00004 per cent formaldehyde the color is distinctly visible but when more highly diluted it does not respond distinctly to the phloroglucin test. See Zeitsch. fur Anal. Chemie, 1900, page 64.—Translators.

these in acetic and sulphuric acids. The results of quantitative analyses by the methods given are unreliable when formalin is present, and hence it is a question if this is the best material to be used even in preserving milk samples that are to be kept some time before being analyzed. (Thompson, Merkel, Kruger.)*

VI. CHROMIC ACID AND OTHER PRESERVATIVES.

Chromic acid, potassium bichromate, as well as ammonium salts and mercury salts, and ammonia, all serve as preservatives of milk samples, but for obvious reasons are used only in samples to be analyzed; 0.25 gram of potassium bichromate is sufficient to prevent the coagulation of 250 c. c. of milk for two months. Chromic acid is used as a 20 per cent aqueous solution. One drop of this in 100 c. c. of milk will keep the latter fresh for eight days; 25 per cent ammonia produces a similar effect. The preserving power of any of these substances is largely dependent upon the temperature of the milk. In no case should it be allowed to rise above 10 degrees C. The results of comparative tests before and after treatment with these preservatives have shown that in the determination of fats, etc., these substances do not interfere.

---

*"Experience of late years in laboratory and anatomical work has abundantly shown that formaldehyde ——— hardens albuminous matter and converts it into a substance with a leathery appearance. ..... Gottstein (Deut. Med. Wach, XXII. 797) determined that such food products when hardened by the use of formaldehyde cannot again be softened by boiling."—Dr. Young, Secretary of State Board of Health of Maine.

The Cornell Experiment Station bulletin No. 118 states: "The behavior in the Babcock test of milk which has been preserved by formalin shows that its composition is in some way affected. ..... When formalin is used the curd often fails to dissolve (in sulphuric acid) and becomes a hard compact mass."

For the effect of formaldehyde on the proteids of milk see also Annalen der Chemie, Vol. 310, p. 25 (1899); Zeitsch. fur Physiol. Chem., 22, 127 (1896); Vermont Experiment Station 11th Annual Report, page 353.—Translators.

VII. SODIUM FLUORIDE.

A one per cent solution of this salt will prevent the souring of milk from three to five days. This strength of solution is usually employed. No practical method has been suggested for detecting this substance.

DETECTION OF ADULTERATIONS.

I. ADULTERATION WITH WATER.

By far the most common adulterant used for milk is water. This adulteration cannot always be detected by ordinary market control tests since, as already stated, the effect of the water which is added is often counteracted by skimming the milk. The result is frequently in such cases that the specific gravity is not essentially different from that of the normal original sample. Sometimes it is possible to discover such admixture by means of chemical reactions. Such tests are based on the fact that in almost every case the water used as a diluent contains considerable quantities of salts such as calcium sulphate and other sulphates and often traces of nitrates which are foreign to pure milk.

Pure milk contains only an exceedingly small amount of sulphuric acid, never more than 0.3 per cent (calculated to ash). In case a considerably larger amount is found, the addition of water is indicated. The test is conducted in the following manner: 50 c. c. of milk are treated with acetic acid to precipitate the casein, filtered, and the filtrate evaporated, dried, and incinerated. The ash is taken up with hot water and a few drops of hydrochloric acid; and the extract filtered, boiled, and treated with barium chloride solution. After heating for some time the resulting barium sulphate is filtered off and washed first with hot dilute hydrochloric acid and then with boiling water to remove the acid. The filter and contents are dried, placed in a weighed crucible, ignited, and weighed.

By subtracting the weight of the filter ash, the weight of the barium sulphate is obtained. One part $BaSO_4$ is equivalent to 0.3429 part SO_3.

For the detection of nitric acid a satisfactory method is to coagulate the milk by means of a solution of calcium chloride free of nitrates, filter off the precipitate, and mix with the filtrate a solution of diphenylamine in concentrated sulphuric acid. In the presence of nitric acid, even of minute traces of it, there appears a blue ring or zone at the line of demarkation of the two liquids.

Egger and Moslinger recommend a solution of 0.02 gram diphenylamine in 20 c. c. of sulphuric acid (1 part H_2SO_4 and 3 parts H_2O) completing the volume to 100 c. c. with concentrated sulphuric acid. Of this solution 2 c. c. are placed in a porcelain evaporator and to it 0.5 c. c. of the milk serum is added. The serum is obtained by heating 100 c. c. of milk with 1.5 c. c. of concentrated solution of calcium chloride and filtering from the resulting precipitate. After adding the serum the material is allowed to stand for a moment or two, then mixed by rotating the dish, again allowed to stand and again mixed. In the presence of only traces of nitric acid a blue streak develops around the edge of the dish and by mixing gradually colors the whole fluid.

Both of these methods for detecting adulteration fail if distilled water containing neither sulphates nor nitrates is used as a diluent. A number of other methods for this purpose have been proposed but they give only approximate results. Some of them, however, are of value to many workers, physicians for example, who often lack time and apparatus necessary for a more exact test. If by one or more of these simple methods, results are obtained which are considered doubtful, as will occasionally happen, the sample may be submitted to a chemist for a more refined chemical analysis.

For the above reason a few of the best processes are here outlined. These apply only to cow's milk.

(1) The method proposed by Lezé and Hilsont is to determine the time required to produce coagulation in a known quantity of milk by the action of rennet. This method gives at the same time a means of judging somewhat as to the freshness of the sample. Upon unadulterated fresh milk at 35 degrees C., rennet reacts in forty minutes when added in the proportion of 1:10,000, that is, one liter of rennet will cause ten thousand liters of milk to curdle in forty minutes when the temperature is 35 degrees. In this process a solution of good commercial rennet is used, one c. c. of it being added to 100 c. c. of milk.

With the above proportions (1:100), pure milk coagulates in three minutes and eleven seconds. When 10 per cent of water is added it requires three minutes and fourteen seconds, and with 50 per cent of water, five minutes and forty-nine seconds. If the time of coagulation exceeds 3 min. 50 seconds, the addition of water or some alkaline preservative is evidenced. On the other hand, if the milk coagulates in less than two minutes it evidently was not fresh, the sudden curdling being due to incipient decomposition. Such milk is unfit for use as food.

(2) For this purpose of detecting the admixture of water to milk, Lescoeur suggests coagulating the sample with rennet and determining the density of the serum at 15 degrees C., and also the total dry matter contained in it. The density of serum of pure milk varies from 1.029 to 1.031, and the dry matter per liter varies from 67 to 71 grams. The addition of four per cent of water diminishes the density about 0.001, and the dry matter about two grams per liter. In making comparisons the following table may be used:

Condition of the Sample.	Density at 15° C	Total dry matter per liter.
Pure milk	1.0300	70.0 grams
100 parts milk + 10 parts water..	1.0275	64.0 "
100 parts milk + 20 parts water..	1.0251	59.0 "
100 parts milk + 30 parts water..	1.0230	54.5 "

(3) By Beckman's method the freezing point of the milk is determined. The results are not influenced by the fats present, but are dependent upon the water content. Normal milk freezes at — 0.554 degrees C. (average). The lowering of the freezing point below that of water is proportional to the concentration. It is lowered about one-half the above number by diluting the milk with an equal volume of water. The sample in question is brought to the freezing point by means of a mixture of ice and salt. A similar test is carried on with a sample of distilled water. The difference in the observed freezing points indicates the quality of the milk. An addition of 10 per cent of water produces a diminution of only 0.055 degrees.

From the results of a large number of analyses an average normal number has been established for the amount of each of the constituents of milk. By comparing other analyses with these figures adulteration may be often exposed. For example, if the fat content of the dried matter in milk is found to be less than 27.6 per cent and the specific gravity of the same less than 1.335, there is but little doubt of falsification (Herz). The nitrogen content of unadulterated milk should never be less than 0.5 per cent;* the ash never less than 0.7 per cent. The relation of ash to the dry matter, minus fat, varies from

*Results of 15,000 analyses.

1:8 to 1:8.5 (Droop-Richmond). Further, in normal milk the ratio of casein to fat should never be greater than 1:1.74 and never less than 1:1.35.* (L. Van Slyke.) Unfortunately it must be admitted that these normal numbers as given will not cover every case without exception. The composition of cow's milk (other kinds of milk need not be considered here) is influenced by so many factors such as climate, breed, feeding, keeping, duration of lactation, etc., so that universal normals cannot be established absolutely.

A never failing proof still remains, namely, the "stall test." In cases of extreme importance a number of samples of milk may be obtained directly from the animals in the stalls. An analysis of these samples furnish data with which results of tests of suspected milk may be compared. Since the fat content and consequently the specific gravity is subject to considerable variation in different samples, much more reliance is put upon the determination of the other constituents, viz., albuminoids, ash, sugar.

II. ADULTERATION WITH STARCH, DEXTRIN, ETC.

Sometimes it is found that skimmed milk is treated with starch in the attempt to restore the original white color. The detection of this adulterant by chemical means is a simple matter.

By acidifying with acetic acid and boiling, the milk is freed from albuminoids. The filtrate obtained is treated with a few drops of a dilute solution of iodine. In the presence of starch the well known blue color appears. This test, however, is no longer to be considered as proof positive of starch. There is in milk and milk products another substance giving a similar reaction. This substance is considered by Herz as an amyloid on account of its similarity to the amyloid discovered by Virchow

*Results of 25,931 analyses.

in pathologic spleen, liver and kidneys. Microscopic tests show that amyloid is similar to starch in size, form and behavior. There is, however, a sharp distinction between them in their reactions with water. By heating with water amyloid does not form a paste such as starch forms. For proving the presence of starch the results of microscopical examinations must be confirmed at least by the iodine and water tests. The same holds good for the detection of dextrin, glue, or gums, sometimes used for adulteration. For these, too much reliance should not be placed upon the results of tests by any one method.

ESTIMATION OF INSOLUBLE FOREIGN MATTER.

RENCK'S METHOD.

A liter of milk is placed in a high, cylindrical vessel and allowed to stand at least two hours, after which time the greater part is removed either by decanting or siphoning. To the rest containing the suspended particles of solid matter, water is added to make up the original volume. This is allowed to stand again for two hours, siphoned, and the process continued until the fluid in the vessel is almost clear and no trace of milk is left. The liquid is then carefully siphoned off leaving only a small amount, without disturbing the sediment. The sediment is then carefully filtered through a filter which has been dried at 105 degrees and weighed. It is washed several times with distilled water, then, to remove any particles of fat contained in the sediment, it is washed with alcohol and ether, and then dried at 105 degrees to constant weight, and weighed. The difference in weight between the clean filter paper and that containing the sediment gives directly the amount of foreign matter in one liter of milk. According to Renck, the milk sold in Berlin averages 10 milligrams to the liter.

In order to remove the dirt and estimate the amount contained in milk bottles used by dairies in delivering

milk, a very simple method is recommended by Stutzer: A heavy test tube (c, Fig. 7) is fastened air tight by means of a piece of rubber tubing (b) to the mouth of a milk bottle, (a). The bottle is inverted and allowed to re-

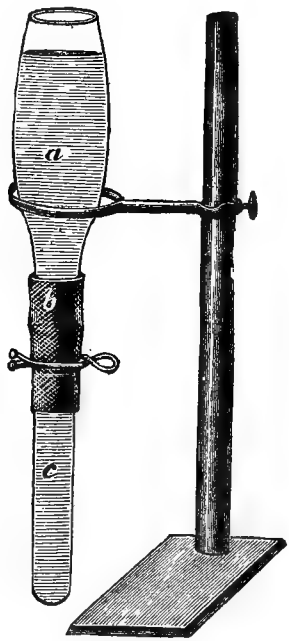

FIG. 7.

main in this position for some time. The particles of insoluble matter settle in the bottom and collect in (c). The connection between the test tube and bottle is then shut off by a clamp, the test tube removed, and the contents treated according to the method described above.

EXAMINATION OF CONDENSED MILK AND CURDLED MILK.

I. CONDENSED MILK.

The examination of condensed milk is made according to the methods described for ordinary milk. A stated amount of the condensed preparation is diluted with a given amount of water and the previously described methods employed. The estimation of dry matter obviously is made with undiluted milk.

To prove the presence of diluted condensed milk in fresh milk use is made of the fact that a part of the sugar is caramelized in evaporation, thereby becoming optically inactive. Therefore the estimation of the quantity of sugar by use of the polariscope gives lower results than the volumetric or gravimetric methods of analysis. Usually the difference between the two methods in fresh milk is never more than 0.15 per cent. (Droop-Richmond.)

II. CURDLED MILK.

Curdled samples are examined according to the usual methods. For the determination of fat the curd is dissolved by adding a few drops of a dilute solution of potassium or sodium hydrate. The specific gravity is best ascertained by a method of Weigul's as follows:

A definite volume of the milk (Vm) is mixed with a known volume of ammonia (Va) of known specific gravity. By shaking, the curd dissolves completely. The volume of the solution (Vs) is equal to Vm + Va. The specific gravity of the solution (Ds) is determined by means of an accurate areometer. From the data thus obtained the specific gravity (Dm) of the curdled milk may be calculated by the formula: $Dm = \frac{Vs \times Ds - Va \times Da}{Vm}$

BACTERIOLOGICAL EXAMINATION OF MILK.

I. INTRODUCTION. METHODS OF PREPARATION. CULTIVATION.

Milk generally contains a large amount of micro-organisms, the number of which varies according to the cleanliness practised in the milking and in the entire management of the dairy. It has been demonstrated that the number of germs present depends upon the amount of foreign matter contained in the milk. From the moment the milk leaves the udder, the bacteria increase very rapidly provided their vitality is not decreased or weakened by heating or by the use of preservatives, so that by estimating the number, an idea can frequently be gained as to the freshness of the milk, as well as the cleanliness of the process by which it is handled. However, bacteria may be contained in the milk even before it leaves the udder, which is the case in certain diseases, the diagnosis of which can be made through a bacteriological examination of the milk. In human milk, which was drawn from a mammary gland, and kept scrupulously clean and under perfectly aseptic conditions by every precaution, various cocci have been found (Honingmann, Lewis, Polleske, Johannessen, Durante, and others).

In examining bacteria of milk the simple dry cover-glass preparation is sufficient in many cases, but the fat must always be extracted. A bit of the milk to be examined is taken up on a loup made at the end of a platinum wire and spread with water upon

a clean cover-glass that is free of all grease, forming a film as thin and even as possible. It is dried without the use of a flame. The cover-glass is then placed in ether for a few minutes, by which process the fat is extracted. It is then taken out and stained in the usual manner. C. Arens combines the two processes of fat-extraction and staining by placing the cover-glass preparation in a chloroform-methyleneblue solution, of the following composition: 12-15 drops of a saturated alcoholic solution of methyleneblue and 4 c. c. of chloroform. It is stained from 4 to 6 minutes and washed.

This simple method is not always successful. The various modifications will be discussed below.

II. QUANTITATIVE ESTIMATION OF GERMS.

For estimating the number of germs contained in milk the gelatine-plate method is employed, using either Koch or Petri dishes. After a stated time the developed colonies are counted with the aid of a counting apparatus. Wolffhugl's counting apparatus is a very convenient device where the Koch plates are employed. As milk usually contains a great number of germs the sample must be properly diluted; more in summer than in winter. Of milk, not rich in germs, 1 c. c. is diluted with 9 c. c. of sterilized water and 1 c. c., equal to 0.1 of this mixture, is then inoculated into 10 c. c. of nutrient gelatine. It is customary to estimate the number in 1 c. c. The estimation of the number of bacteria may serve as an indication of the quality of the so-called sterile milk in commerce. Milk is seldom rendered germ-free, for by so doing serious chemical changes very often take place effecting the appearance, taste, agreeableness and digestibility.

When the plate culture method is used in isolating and growing the different bacteria contained in the milk, dilutions of the original inoculated tubes must be made in the usual way.

III. MILK ANOMALIES.

The so-called disease of milk, or "milk anomalies," often have their origin in the action of the presence of micro-organisms. The most important of these anomalies are the following:

1. Red Milk. This is caused by different saprophytic (non-pathogenic) genii of bacteria: Sarcina species, Bacillus prodigiosus, Bacillus lactis erythrogenus, Bacillus rubidus, Spirillum rubrum, Micrococcus cinnabareus, and by a red yeast.

(a) Of the Sarcina, the most common are Sarcina rosea Menge, Sarcina rosea Schrotter and Sarcina aurantiaca. The different species, of which numerous germs are found in the air, are distinguished from one another by their readiness or slowness in liquefying gelatine. Aurantiaca does not liquefy gelatine, and grows very slowly. All grow upon the ordinary culture media and are strongly aerobic.

(b) The Bacillus prodigiosus is a very small, short rod, possessing no motion. It grows as well at room temperature as at 37 degrees and liquefies gelatine. At incubation temperature there is no pigmentation, the latter condition going hand in hand with the production of trimethylamine (odor like that of pickled herring).

A dark red color is beautifully shown upon potato culture; this becomes light red when treated with acetic acid. Ammonia restores the original deep red color. Sterile milk inoculated with Bacillus prodigiosus slowly precipitates the casein; the serum-zone beneath the layer of cream gradually becoming blood red.

(c) Bacillus lactis erythrogenus (Hueppe) liquefies gelatine. In bouillon and solid culture media it produces a yellow pigment, while in milk and whey the pigment produced is red. The red color is impaired by light and acids.

(d) The Bacillus ruber is a long, thread-like, very motile rod, which when isolated is grouped in threes or fours and occasionally shows from two to four spores. Upon agar a yellow layer is formed. It liquefies gelatine. Potato cultures are covered with a rusty red film.

(e) Spirillum rubrum is a thick, clearly transparent bacterium, having a regular spiral movement and is provided at each end with a flagellum. The long spirilla exhibit slow motion while the short ones are actively motile. Reproduction takes place by division. Spore formation is doubtful. The spirillum grows between 16 degrees and 40 degrees, best at 37 degrees. Upon gelatine the growth is very slow, non-liquefying. In plate cultures many small, grayish-red colonies the size of a pin head are formed. In stick cultures, round, reddish granular colonies are formed along the track of the needle. Upon agar and blood serum a grayish white, sharply defined layer is first developed, later producing thick, rosy-red layers. Upon potato a moist glistening layer, reddish in color, is formed.

(f) Micrococcus cinnabareus is a large coccus often in the form of a diplococcus and often grouped in threes and fours, and does not liquefy gelatine. It grows very slowly. Upon plates, after about four days, the deep colonies appear as pale brick colored points. In gelatine stick cultures, white colonies are seen along the line of punctures and upon the surface rose-colored buttons develop, becoming dark red in time.

(g) Sometimes the red color of the milk is due to coloring matter contained in the fodder or blood.

2. *Yellow Milk.* Yellow milk is caused by the Bacillus synxanthus (or Bacterium synxanthus Ehrenburg). The micro-organism develops as an actively motile small rod, which, when inoculated into sterilized milk, gives it a citron yellow color. The yellow coloring matter disap-

pears under the action of acids and is restored by neutralizing the acid with an alkali. It is insoluble in alcohol and ether. Yellow milk has a sickening odor and taste.

3. *Blue Milk.* The Bacillus cyanogenus, Bacillus cyaneofluorescens Zangemeister, and Bacillus janthinus, all impart a blue color to the milk.

(a) The Bacillus cyanogenus or Bacillus syncyaneus is a small rod frequently grouped in twos. Numerous flagella on the sides give it a vigorous motion. The bacillus grows readily on slightly acid media, best at room temperature. Gelatine plate cultures show superficial colonies and stick cultures exhibit dark gray layers. It grows well on media containing grape sugar and glycerine. Sterilized milk becomes slightly alkaline and blue gray. Unsterilized milk becomes dark blue. This bacillus does not otherwise tend to decompose the milk. Its activity being dependent upon the formation of pigments.

(b) Bacillus cyaneofluorescens Zangemeister differ very sharply from the above. It is short and oval in shape, motile, does not liquefy gelatine, but gives it a yellowish green fluorescence. Upon sugar gelatine it grows as a white film. The cultures have a strong odor of trimethylamine. Unlike Bacillus cyanogenus, it does not effect sterilized milk, but gives it a blue color when inoculated together with the Bacillus acidi lactis.

(c) Bacillus janthinus, a rod, grows on gelatine plates as a white film, gradually becoming violet at the border. The stick culture is violet only on the upper surface. On potato a violet colored layer is formed. Sterilized milk shows blue spots on the cream-surface. After a short time the casein is precipitated, an alkaline reaction is shown and an abundant amount of ammonia is formed.

4. *Stringy Milk.* The micrococcii of Schmidt-Muhlheim give milk a slimy property, capable of being drawn

out into threads. The bacteria of Duclaux and bacteria lactis viscosis Adametz do likewise. The latter flourishes on all media, especially well upon peptonized glycerine gelatine. It causes sterilized milk to become threadlike after three or four weeks. The Micrococcus viscosus of Schmidt-Muhlheim grows in garland-chains and produces a slime similar to plant-slime. Contrary to many other slimy fermentation processes caused by micro- organisms, no carbonic acid is found. More definite data concerning the cultivation of the Schmidt-Muhlheim coccus is lacking.

5. *Slimy Milk.* Micrococcus viscosus, a bacillus found by Weigmann and Zirn and by G. Leichmann, does not render milk ropy, but slimy and soapy.

(a) Micrococcus viscosus grows in chains and gives sterile milk a gummy, slimy viscosity.

(b) Weigmann isolated from slimy, soapy milk, four species of bacteria, one of which gives to sterilized milk the above mentioned peculiarities, while the others merely produce a yellow coloring. The species in question produces a slimy precipitate and an alkaline reaction, the precipitate disappearing after a short time, the milk becoming watery and fluorescent.

(c) Leichmann's bacillus is a slender rod with rounded corners, single or in pairs, seldom lying in chains, which decomposes sterilized milk up to 50 degrees, developing considerable gas and causes the whey to become slimy.

6. *Bitter Milk.* Proteus vulgaris Hauser, Bacillus von Bleisch, Bacillus and Micrococcus liquefaciens lactis amari Freudenreich, the bacteria of bitter milk of Hueppe, Flugge (as below), also a number of bacteria found by von Sterling, produce bitter milk. The bitter taste is the result of the formation of peptones, which is found in the serum of the peptonized milk. By treating the same with potassium or

sodium hydrate and a few drops of diluted copper sulphate solution, it produces a violet to a rose-red color, the reaction for peptones (biuret reaction).

So-called "*poison milk*," which acts like alkaloids upon animal organisms, is probably due to the presence of ptomaines or toxins produced by bacteria from the albuminoids of the milk.

To obviate the above described milk anomalies, which are mostly the results of insufficient cleanliness, the stalls must be thoroughly cleaned with water, the walls frequently white-washed, all utensils boiled, the udder carefully washed with warm boiled water and the persons entrusted with the milking must exercise perfect cleanliness.

Of the numerous micro-organisms contained in nearly all milk, the peptonizing species, the so-called bacteria of bitter milk, "Hueppe's" demand special attention. To this class belong a number of strongly anaerobic and 12 facultative anaerobic species, recently described by Fluegge. Under these, according to Fluegge, probably come Bacillus mesentericus fuscus and vulgaris, Bacillus liodermis, Bacillus albus lactis Loeffler, Bacillus butyricus Hueppe and Botkin, the bacteria of Duclaux, the Kreuger bacillus, Proteus vulgaris, Micrococcus Conn, Clostridium foetidum and butyricum, Bacillus muscoides Liborius, and four varieties called by Sterling, bacterium lactis peptonaus. Fluegge has recently published the results of a study of the bacteria which peptonize milk, or rather the casein of milk and which for the largest part belong to the group of hay and potato bacilli. He has also called attention to their dangerous character. These bacteria are characterized by forming spores which have extraordinary powers of resistance and which are killed only when exposed for two hours to the influence of steam at 100 de-

grees C. and which therefore retain their full vitality in milk sterilized according to the usual method. Moreover, their presence is unnoticed in fresh milk kept at ordinary temperature or in boiled milk; their influence, however, is noticed when imperfectly sterilized milk is kept at 37 degrees in an incubator. Such milk, or sterilized milk inoculated with pure cultures of peptonizing bacteria, shows profound changes after standing from one to five days. Beneath the cream zone a transparent zone is formed which appears to be made up only of serum. Gradually the transparent zone becomes wider, the casein not yet peptonized begins to separate into flakes, and in most cases rennet fermentation occurs in addition. Milk so changed, has a bitter, pungent, peptone-like taste. The identification of individual species presents great difficulty. It is not possible to recognize them through cultures upon agar and gelatine plates, but identification is usually successful through cultivation upon potatoes, absolutely sterile skimmed milk, alkaline gelatine, and stick culture in sugar agar. Often these methods fail and it is necessary to study the metabolic assimilation and the thermal death point of the germ to assure an accurate diagnosis.

(An accurate and detailed description of all the characteristics of the bacteria mentioned above is found in "Fluegge's Zeitschrift fuer Hygiene and Infectionskrankheiten, Vol. XVII, 292.")

Abnormal milk with neutral or alkaline reaction frequently becomes curdled, due to the presence and the action of the bacteria just mentioned. Normal sour curdling is caused by a series of widely diffused kinds of bacteria, viz: the Bacillus acidi lactici Hueppe; Bacterium acidi lactici Grotenfeld; Bacterium limbatum acidi lactici Marpmann; Bacillus acidi lactici Leichmann; Micro-

coccus Marpmann; Sphaerococcus Marpmann; and Streptococcus acidi lactici Grotenfeld. The difference between the individual species lies chiefly in their physiology. For example, Bacillus acidi lactici Hueppe, produces lactic acid and carbonic acid, and the Bacillus acidi lactici Leichmann, lactic acid and ethyl alcohol, etc.

IV. DEMONSTRATION OF INDIVIDUAL PATHOGENIC BACTERIA IN MILK.

1. BACILLUS TUBERCULOSIS.

Sometimes the cover-glass preparation is successful in demonstrating the bacteria. For the preparation of such, different directions are given.

(*a*) *Method of Ahrens:* A drop of milk is placed upon the cover-glass with a drop of sterilized water. This is dried in the air and fixed by slightly warming. The cover-glass is then placed in a mixture of 3 drops of a concentrated alcoholic solution of fuchsin and 3-4 c. c. of chloroform. After ten minutes it is transferred to 4-6 c. c. of 96 per cent alcohol, to which has been added 2-3 drops of diluted sulphuric acid. After washing in water it is double-stained with an aqueous solution of methylene blue.

(*b*) *Method of Alessi:* A drop of milk is dried upon the cover-glass by moderate warming and 2-3 drops of one per cent sodium hydroxide solution added. It is again warmed until saponification of the fat takes place (three or four minutes), washed with water and stained as usual, for example, according to Gabbet; 2 minutes in cold carbolic fuchsin, 50 seconds in cold sulphuric acid methylene blue Gabbet, i. e., methylene blue 2 parts, 25 per cent H_2SO_4, 100 parts.

(*c*) *Method of Schrank:* A drop of diluted milk is dried in the air on a cover glass. The casein is fixed by placing the cover-glass on a copper

sheet and heating it without producing a brown color. It is then placed in a dish containing 4 c. c. of ether or chloroform, shaken until the fluid is evaporated and stained as usual. If with these processes the results are negative, the attempt must be repeated with a larger amount of milk. One hundred grams of milk are mixed with about 5 c. c. of sodium hydrate and shaken with a large amount of ether in a separatory funnel until no more fat goes into the solution. A sample of the ether evaporated will convince one of this fact. The fat-free liquid is brought into a funnel shaped glass or a sediment apparatus and allowed to stand for 24 hours. After this time a dry preparation of a sample of the sediment is made after the manner described.

(*d*) *Method of Thoerner:* 20 c. c. of milk mixed with 1 c. c. of 50 per cent potassium hydrate are placed in centrifugal tubes and then placed in boiling water for about two minutes until the milk becomes yellowish-brown. They are then removed and well shaken, the mixture treated with 20 c. c. of glacial acetic acid, again thoroughly shaken, placed in boiling water for three minutes and centrifugalized 10-20 minutes. The liquid above the sediment is drawn off and the sediment with about 40 c. c. of hot water again centrifugalized for ten minutes, and a cover-glass preparation made from the sediment in the usual manner.

(*e*) *Method of Ilkewitsch:* 20 c. c. of milk are coagulated with citric acid, and filtered. The residue on the filter is dissolved in a very dilute aqueous solution of sodium phosphate, mixed with about 10 c. c. of ether and shaken for 10-15 minutes. The solution found beneath the fat zone is drawn off, centrifugalized, and a preparation of the sediment made on a cover-glass.

The cultivation of the tubercle bacilli from milk is quite difficult. They grow excluded from air and light

only upon solid culture media of cattle blood serum and glycerine agar. They grow remarkably slow, and, in spite of great dilution of the milk, a sample from which the inoculation is made, the media become very quickly overgrown by the rapid and luxurious growing saprophytic bacteria of the milk. For the determination of the tubercle bacilli the solid culture media are not so well adapted as the fluid ones; of the latter, especially the Nocard bouillon (nutrient bouillon with 6 per cent glycerine and 3 per cent gelatine). A large number of samples are suitably started and kept for at least three weeks at 37 degrees in an incubator. From the colonies developed in one manner or another, microscopic preparations are made and stained in the usual manner.

More accurate information is gained by experimenting on living animals to determine the presence of tubercle bacilli in the milk, or whether their number is sufficient when the milk is consumed to produce tuberculosis. Guinea pigs and also rabbits, are fed with the suspected milk and after 8-10 weeks examined for tuberculosis of the intestines, mesenteric glands and the liver. Instead of feeding, intraperitoneal injections can be made. The sediment secured from centrifugalizing impure milk is especially adapted for this purpose. The presence of tuberculosis is diagnosticated by sections of the dead or slaughtered animals.

2. THE CHOLERA BACILLUS.

The demonstration of it presents great difficulty and must be done according to the proper methods of examining water. Neither staining, appearance, nor animal experimentation are especially characteristic. Its identity can be confirmed only through cultivation.

For the diagnosis of the cholera bacillus a method

given by Koch may be used. Stock cultures are prepared. In the meantime the material to be examined is put in an alkaline 1 per cent peptone solution and kept in an incubator at 37 degrees. Within 6 to 12 hours the cholera bacilli, if present, will have rapidly increased, gathered at the upper surface of the fluid, at times forming a fine film (on account of the great need of oxygen). From the upper surface of the peptone solution, or in other words, from the thin film, agar and gelatine cultures are inoculated. After twelve hours characteristic growths and reactions appear; best on agar at 37 degrees and on gelatine at 22 degrees. Regarding the latter point a complete text-book of bacteriology must be referred to. It may be mentioned that the cholera bacilli grow only upon alkaline culture media, and liquefy blood serum. They give a cholera red indol reaction and have a remarkable tendency to dry up.

3. THE TYPHUS BACILLUS.

The Typhus bacillus is equally as difficult to demonstrate in milk as is the exciter of cholera, particularly since it is easily grown over by species very similar to it and most always occurring together with it, for instance, by the Bacillus coli communnis.

For the differentiation of these two micro-organisms, one utilizes, besides the different reactions with their pure cultures (indol reaction, behavior towards sterile milk, etc.), a culture process (method given by Elsner) upon plates of potato gelatine mixed with 1 per cent potassium iodide. Upon this media colonies of Bacillus coli communnis appear after 24 hours as yellowish white points, while at this period the Typhus bacillus will not have grown. After 48 hours the Typhus Bacillus forms small, bright glistening buttons (knobs), while Bacillus coli communnis forms large brown spots (specks).

For the preparation of the Elsner culture medi 500 grams of potatoes are extracted with a liter of water; to this extract 100 grams of gelatine are added; 10 c. c. of the potato gelatine is made alkaline with 2.5—3 c. c. of 1-10 normal sodium hydroxide mixed with 1 per cent potassium iodide.

The methods of cultivation of the known species, as well as the possible occurring pathogenic staphylococcus, and streptococcus, the streptococcus of infectious induration or the Streptococcus agalactiae contagiosae—the cause of chronic and acute mastitis of the cow—the Staphylococcus and Streptococcus Guillebeau, the bacillus Guillebeau, etc., need no special explanation; they are governed by the universal technique of bacteriology which has heretofore been mentioned.

The Bacillus Guillebeau is a facultative anaerobic motile germ, gas forming, which does not liquefy gelatine. In stick cultures it grows needle-like with finely pointed iris knobs. It causes sterilized milk to coagulate. Upon potatoes white or brown layers are formed. According to Grame the bacillus does not stain. In Guinea pigs and rabbits it is not pathogenic. Inoculated into the udder it produces mastitis.

The Staphylococcus mastitis Guillebeau, according to Grame, is stainable and shows motility. It is facultative anaerobic, without gas development, liquefies gelatine with the formation of a sediment and causes sterilized milk to coagulate. Upon potato a yellowish white layer is formed. The Streptococcus mastitis sporadicae Guillebeau is also facultative anaerobic, liquefies gelatine and thrives only very scantily upon potato. By inoculation it shows no pathogenic peculiarities.

APPENDIX.

EXAMINATION OF POWDERED, MALTED MILK AND OTHER SOLID FORMS OF PRESERVED MILK.

The examination includes the determination of dry matter (water), ash, phosphoric acid in the ash, the detection in the ash of injurious metals entered in packing, fat, albumin, the amount of soluble matter in water (extract) and carbohydrates (sugar and starch). An average sample is used.

1. The dry substance is determined according to the directions for milk (Page 28). Ten grains of the material are usually taken for the test.

2. The usual methods hold good for the ash and phosphoric acid. In the ash it is important, when metal boxes are employed for packing, to test for arsenic, antimony, lead and tin.

3. Fat: 10 grams are extracted with ether in the Soxhlet extraction apparatus, the ether fat solution evaporated and the remaining fat weighed. (Page 31.)

4. Extract: 25 grams are extracted with hot water and this repeated until nothing more goes into the solution. The entire amount, with the aid of a funnel provided with a perforated base (Witt plate), is filtered through mull or muslin. The aqueous extract (filtrate) collected is brought to a stated volume (1 liter as a rule) cooled off and an aliquot part, about 50 or 100 c. c. evaporated to dryness upon the water bath in a platinum

(or porcelain) dish at 105 degrees until dried to a constant weight, then weighed and the results reckoned on the whole fluid mass.

5. Carbohydrates: From the solution saved from the extract determination, a measured amount, about 200 c. c. is mixed with 5 grams of concentrated H_2SO_4 (or 18 c. c. of concentrated hydrochloric acid) and boiled 5 hours in a flask provided with a reflux condenser.* Thereby all the carbohydrates are inverted, that is, they are transformed into reducing sugars and can be determined according to methods given for milk sugar. The inverted cooled solution is brought at room temperature to a stated volume, an aliquot part of the same taken and treated as for the milk sugar determination given on page 50.

*Such prolonged heating at such high temperature is usually inadvisable and unnecessary. The common practice at present is to heat for 15 minutes at 68°—70° C. See bulletin No. 46, U. S. Dept. of Agr., Div. of Chem., p. 31.—Translators.

BIBLIOGRAPHY.*

I. BACTERIOLOGY OF MILK.

Bacteria in Milk and Its Products. Storrs Agr'l Exp. Sta. Bulletin No. 4, by H. W., Conn. (1889).

Bacteriological Examination of Milk. By J. Clauss (Diss. Wurzburg. 1890, p. 518).

Bacteriology of Normal and Abnormal Milk. By L. Adametz (Nos. 1-3. Oesterreich. Monatssch. fur Thierheilkunde. 1890).

The Effect of Centrifugal Action on the Distribution of Bacteria in Milk. By Scheurlen (Arb. a. d. Kais. Ges. Amt. No. 7. 1891).

The Micrococcus of Bitter Milk. The Ripening of Cream. Conn, Storrs' Exp. Sta. Third Annual Report (1891).

Bacteria in the Dairy. Conn, Storrs' Exp. Sta. Rept. for 1891.

Bitter Milk and the Sterilization of Milk by Heating Under Exclusion of Air. By M. Blisch (Ztg. Hyg. No. 13, p. 81. 1891).

The Sterilization of Milk. (Milch. Ztg. 1893).

The Sterilization of Milk on a Large Scale. (Ztg. Hyg. No. 13, p. 42.)

The Spread of Contagious Diseases in Milk and Dairy Products. (Molk Ztg. No. 8, 1893.)

*No effort has been made to make this bibliography complete or inclusive. Only a few of the more important recent researches, bearing directly upon the subject matter of this book, are here collated.—Translators.

The Danger of Consuming Milk of Sick Cows. By J. F. Baum. (Arch, Wiessensch. u prak. Tierheilkunde. Vol. 18, No. 3-4.)

Some Bacteriological Work in the Dairy. By L. H. Pammel. Iowa Sta. Bulletin No. 21.

On Ropy Milk and the Source of Bacteria in Milk. (Jour. of the American Chem. Soc'y. Vol. 15, No. 6.)

A Review of Recent Work on Dairy Bacteriology. By E. W. Allen. (Exp. Sta. Rec. Vol. 5, p. 1042.)

Behavior of Cholera Bacilli in Milk and Cheese. By H. Weigmann & G. Zirn. (Landw. Wochenbl. Schles. Holst. Milch. Ztg. 23. 1894. No. 20, pp. 311-313.)

Behavior of Cholera Germs in Milk. By H. Weigmann. (Milch. Ztg. 23 (1894), No. 31, pp. 491-493.)

Effect of Milk on Cholera Bacilli. By W. Heese (Ztschr. Hyg., 17 (1894), p. 238; abs. in Chem. Ztg., 18, 1894.)

Dairy Bacteria.. U. S. Dept. of Agr., Office of Exp. Stas. Bulletin No. 25. By H. W. Conn.

Bacteriological Investigation in the Dairy. By G. J. Leufven. (Rept. Ultuna Agl. Inst., 1894.)

The Source of the Bacteriological Infection and the Relation of the Same to the Keeping Quality of Milk. Wis. Exp. Sta. Rept. for 1894. By H. L. Russell.

The Bacteriological Contamination of Milk. Ont. Agr. Exp. Sta. and Coll. Rept. for 1896. By F. C. Harrison.

The Persistency of Bacteria in the Milk Ducts of Cow's Udder. By G. S. Ward. (Jour. of Appl. Micros. No. 1, 1898.)

Bacillis Typh. Abdominalis in Milk and Butter By H. F. Bolley and M. Field. (Backtuper to. Abt. No. 24, 1898.) (Centbl. Bakt. u. Parisit., No. 24, 1898, pp. 881, 887.)

II. PRESERVATIVES AND ADULTERATIONS. METHODS OF DETECTION.

The Influence of Milk Preservation. By A. W. Stokes, Analyst. No. 16 (1891).

The Preservation of Milk in Norway with Boric Acid. Weigmann. (Milch Ztg., 1893.)

The Aeration of Milk. Ind. Exp. Sta. Bulletin No. 44. By C. S. Plumb (1893).

The Recognition of Foreign Fats in Butter. By W. Johnstone. (Abs. in Chem. Centbl., 1893.)

Note on the Detection of Adulteration of Fresh Milk with Diluted Condensed Milk. By D. Richmond, Analyst. (No. 18, 1893.)

Creaming and Aerating Milk. By H. H. Wing. (N. Y. Cornell Sta. Rept. for 1892, pp. 113-142.)

Notes on the Detection of Formalin. By H. D. Richmond and L. K. Bosly, Analyst. (No. 20.)

The Preservation of Milk and Cream for Household Purposes. (The Nursery of the Sick Room.) By R. Bell. (No. 86, p. 13, 1896.)

The Method of Distinguishing Pasturized and Unpasturized Milk. By V. Storch. (Bericht. des. Veruchslaboratoriums der, Kgl. Veterinar—und Lardbanhochschule. Copenhagen. No. 40, 1898.)

Effect of Formaldehyde of Milk. Analyst, Vol. 20, p. 157.

III. VARIATION IN THE COMPOSITION OF MILK.

The Analysis of Milk of Different Breeds of Dairy Cows. Mich. Exp. Station, Bulletin No. 68. By G. H. Witcher. (Feb. 1890.)

The Effect of Food Upon the Quality of Milk. Iowa Sta. Bulletin No. 14. By J. Wilson (1891).

The Curdling of Milk During Thunder Storms. By T. O. Lemei. (Abs. Milch Ztg. No. 20, 1891.)

The Effect of the Change of Quarters Upon the Quantity and Quality of Milk. Vt. Exp. Sta. Rept. for 1891. By B. L. Hills.

Abnormal Milk. By R. G. Smith. (Jour. Soc. Chem. Ind., 13 (1894), No. 6, pp. 613 and 614.)

Conditions Affecting the Consistency of Milk. Wis. Exp. Sta. Rept. for 1896. By S. M. Back and H. R. Russell.

IV. DETERMINATION OF FATS.

The Babcock Method of Determining Fat in Milk and Cream. Conn. Exp. Sta. Bulletin No. 106 (March, 1891).

Method of Determining Milk Fat. Penn Sta. Bulletin No. 12. By B. W. Frear and Glholter (1890).

A New Method for the Estimation of Fat in Milk, Especially Adapted to Creameries and Cheese Factories. Wis. Exp. Sta. Bulletin No. 24. By S. M Babcock (1890).

A Discussion of Cochran's Method for the Determination of Fat in Milk for the Use of Dairymen. N. Y. Agr. Station Bulletin No. 17. By G. S. Caldwell (1890).

A Method for the Determination of Fat in Milk and Cream. N. Y. State Exp. Sta. Bulletin No. 19. By P. Collier. (Illustrated.)

A New Milk Test. Vt. Exp. Sta. Bulletin No. 21 (1890).

The Importance of Extraction of Paper Coils in Milk Analysis by the Adams Method. Bulletin No. 16. By P. Vieth, Analyst (1891).

A Comparison of the Methods of Determining Fat in Milk. By L. F. Nilson. (Chem. Ztg. No. 15.)

Determination of Fat in Sour Milk. By Mekenberg. Chem. Ztg. No. 15.)

A New Method of Determining the Amount of Butter

Fat in Milk. Miss. Exp. Sta. Bulletin No. 21. By L. G. Paterson (1892).

The Best Milk Tester for the Practical Use of the Farmer and Dairyman. Colo. Station Bulletin No. 20. By Walter J. Quick (1892).

The Acid Butyrometric Method of Rapidly Determining Fat in Milk. (Chem. Ztg. No. 98, 1892.)

The Estimation of Solids and Fat in Milk. By J. B. Kinnear. (Chem. News, No. 68, 1893.)

The Recognition of Foreign Fats in Butter. By W. Johnstone. (Abs. Chem. Centbl. 1893).

Remarks on Babcock's Method of Fat Determination. By F. W. Woll. (Tidskr. norske. Landbr. No. 1 (1894), pp. 285-292.)

V. COMPOSITION.

The Constituents of Milk. Wis. Exp. Sta. Bulletin No. 18. By S. M Babcock (1899).

The Analyses of Milk of Different Breeds of Dairy Cows. Mich. Exp. Sta. Bulletin No. 68. By R. C. Kedzie (1890).

The Analysis of Milk. N. Y. Cornell Sta. Bulletin No. 25. By H. Snyder (1890).

Composition of Cream and Butter Milk, and the Loss of Butter Fat in Churning. Conn. Exp. Sta. Annual Report (1891).

Analysis of Milk and Other Dairy Products. Miss. Sta. Rept., 1891.

Composition of Dairy Products. Minn. Sta. Bulletin No. 27. By H. Snyder.

Composition of Milk and Milk Products By H. D. Richmond, Analyst. (Mar., 1893.)

Citric Acid and Phosphate of Lime in Solution in Milk. By L. Zaudin (Ann. Inst. Pasteur, 8 (1894). No. 17, pp. 502-505.)

The Chemical Composition of Cow's Colostrum. By L. Vaudin. (Bul. Soc. Chem. Paris, 11-12 (1894), No. 13, pp. 623-625.)

Frozen Milk. (Milch Ztg. No. 26, 1897.)

Constituents of Milk. Wiley, 464.

Composition of Milk of Different Mammals. Wiley, 465.

Milk, Skim Milk, and Whey; a Study of Their Comparative Composition and Specific Gravity. By C. B. Cochran. (Jour. Amer. Chem, Soc., Vol 15, p. 347.)

VI. METHODS OF ANALYSIS.

Investigation of Milk Tests. Ill. Exp. Sta. Bulletin No. 10. By E. H. Farrington (1890).

Determination of Casein in Milk. By Jroux. (Chem. Centrabl. No. 1, 1891.)

The Determination of Nitrogen by the Kjeldahl-Jodlbam Method. (Exp. Sta. Record. Vol. 5, p. 462.)

Saponifying Butter Fat by the Leffmann and Beam Method. Del. Sta. Rept. for 1892. By C. F. Penny.

Contribution to Milk Analysis. By E. Beckmann. (Milch Ztg. 23 (1894), No. 44, pp. 702-703.)

Milk Analysis. By Lescouer. (Abs. in Chem. Centbl., 1894, II., No. 19, pp. 816).

Examination of Composite Samples of Milk. By M. Weibull. (Chem. Ztg. 18 (1894), No. 81, p. 1567, 1568.)

Method of Estimating Acidity of Milk. By M. Schaffer. (Staz. Sper. Agr. Ital., 26 (1894), pp. 164-167; Abs. in Jour. Chem. Soc. 67-68, 1895.)

The Determination of Specific Gravity of Clabber Milk. By Rischloff. (Milch Ztg. No. 48.)

A Method for Determining the Acidity of Milk. By A. Devarda. (Milch Ztg. 1896.)

Importance of Expelling CO_2 in Determining the Acidity in Milk. Bulletin No. 47, Div. of Chem., U. S. Dept. of Agr. p. 127.