THESIS

ON

MILK ANALYSIS

FOR

DEGREE OF B S

BY

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'83

N2

Milk and Its Analysis

Especially the application of the Centrifugal Machine and the variations in creams.

Milk, which forms one of our principal foods and enters into the preparation of most articles of diet has long been more or less of a problem for chemists and sanitary officers. There is perhaps no article of food which is so commonly adulterated as is the milk sold in the large cities of the world. Yet the difficulties which surround the examination make all the processes more or less impractical. Really there is but one difficulty, the great number of samples it is necessary to examine. To make a complete analysis of each sample would require an enormous corps of assistants and more apparatus than the economy of most of our City Fathers would allow. So it has happened that different chemists have taken some one constituent of milk as a basis by which to standardize the milk.

The constituents of milk are --

Water	Casein		Potash	
Milk Sugar	Albumen	Casein	Soda	
Fat	Nuclein		Lime	
	Lacto-protin		Magnesia	Ash.
			Phosphoric acid	
			Chlorine	

Wanklyn in his excellent treatise has chosen "Solids not fats" as the most invariable constituent and therefore the best fitted to reveal adulteration. Merchand would require a certain percent of cream, but this, as will be shown later, failed to show whether sophistication has been practiced or not. Ladé would determine the amount of casein by a standard solution of mercurious nitrate and Monier and Wanklyn suggest the determination of the same by a solution of Potassium Permanganate. Reveil and Chevallier suggest the determination of lactose by means of the Polariscope. Why some have failed to make the percentage of ash the basis I do not know, for it

shows no wider variations than do the percents of other components Commercial milk is the mammary secretions of the cow and is an aqueous solution of casein, milk sugar, and a small amount of mineral matter and suspended in it are a great number of minute fat globules, which when they rise to the surface form the cream. The following is the constitution of milk, as given by Wanklyn and found in my own analyses.

	Country fed	Town fed	My Own
Water	87.561	85.922	85.987
Fat	3.07	4.03	3.47
Casein	4.04	5.07	3.22
Sugar	4.62	4.30	3.97
Ash	.709	7.38	.419
Solids not fat	9.369	10.048	7.609.

My analyses represent six different breeds including the Grade and Blooded Holestein which have increased the percent of casein. The conditions of feed of most of them differed.

The ash in my analyses is simply the soluble ash the insoluble has not been deducted from the casein.

The fat of milk, often erroneously called oil, is a mixture of mineral fats, principally olein, stearine, palmatein and a small quantity of butyrine. All of these ethers of Glycine are represented by the general formula $a(C_n H_n O)_3 \}^{C_3 H_5} O_3$. The fat is soluble in ether and its melting point is about 37°. It is supposed that these oil globules are covered by a thin membrane. Some claim that in the process of butter making this coat is disolved by the free ~~by the~~ lactic acid formed, but the following experiments entirely overthrows this. To 50 c.c of milk in a flask of 200 c.c. I added a few drops of lactic acid. To another sample of the same milk $Na_2 CO_3$ on shaking for fifteen minutes the butter was seen to form as readily in the one flask as in the other. Although Baumhauer thinks that this, also disproves the idea of any coat at all I cannot see why it should. Under the head casein is included all the nitrogenous constituents of milk, as these are generally

estimated together. Caseine is peculiar to milk. It is easily precipitated from its solution in milk by tannic acid rennet and many of the mineral salts and acids. When precipitated by an acid it should first be largely diluted with water and the acid added drop by drop. When thoroughly washed with water, alcohol and ether, the caseine obtained is white and brittle. There has always been more or less dispute as to the nutritive properties of milk. Some have claimed that the proportion of cream alone should determine it and that skim milk is of little value. While it is true that the percent of cream does add largely to the nutritive value of milk, yet the idea is fast becoming prevalent that skim milk has its uses too. There are 43.75 grams of nitrogen in each pint of whole milk. In accordance have arisen the various ideas of "pure milk". One city requires a certain percent of fats, another of "solids not fats" but it seems most natural to take the latter, as the variation is so much less. According to Edward Smith

10 grms. of new milk when consumed in the body produces an amount of heat sufficient to raise 1246 pounds one foot. He further more says that new milk is more nutritive than flesh and skim milk equal to it. Calves fed on skim milk thrive very well, and the most extensive and successful stock raisers in Iroquois Co., feds his calves on skim milk from the time they are five days old. I think it is useless to contend that the nutrition of milk is due entirely to the amount of cream. But nevertheless, milk as used in most of our families is bought mostly for the cream that rises and skim milk is often thrown away and for them the commercial value of the milk depends on the amount of cream that will rise without reference to whether it is adulterated with water or not For family use a watered milk, with ten percent of cream is better, than a pure milk with five percent of cream. The Milk sugar $C_{12} H_{22} O_{11} H_2O$ is of less importance than the other two. It differs much from cane sugar in

solubility Sp. Gr. (1.53 while cane sugar is 1.60) and behavior toward the alkaline copper solution, Polarization of light etc. It may be estimated the same as grape sugar except that the proportions are different.

The ash is of the least importance unless we are determining adulterated milk, it should be white contain no trace of sulphates, no alkaline carbonates and no excess of chlorine. Mainly it is the phosphate of lime. Its composition varies slightly with the food of the cow. The following composition given by

	Blythe	Smith	Myself.
K_2O	18.82	32.09	20.35
Na_2O	11.58	9.49	14.01
CaO	22.77	17.34	24.75
P_2O_5	27.03	28.70	25.15
MgO	3.37	2.20	2.68
Cl	16.23	9.72	12.35
	100.14	99.54	99.29

Of the water nothing need be said. Of milk in general it may be said, it is a white emulsion like fluid with a sweetish pleasant taste, its Sp. Gr. is 1.028 to 1.032 When fresh it is slightly alkaline, but soon becomes acid owing to the lactic fermentation. Its opacity is due to the minute oil globules of about 1/2500 of an inch in diameter held in suspension. These dissolve readily in the caustic alkalies

Cream is the scum which rises to the surface owing to its low specific gravity. The upper strata is always the richest in fat and the darkest in color. Sp. Gr. is about 1.0.244. Its composition varies very much as may be seen from the results of my experiments tabulated father on. Both heat and fermentation favor the rise of cream. "Cream rises most rapidly at a temperature about 4° to 6° C." Skim milk is a little heavier than new, its Sp. Gr. is 1.0378. It has a bluish tinge and undergoes fermentation very easily.

"Its composition is.

H_2O (Water)	88	But like the others it
Casein	4	varies very much"
Sugar	3.80	Smith."
Fat	1.80	
Ash	.80	
	78.40	

The analysis of milk is easy, a little skill and practice however are quite essential especially as the factor, time, is to enter so largely into it. It is very necessary to get an average sample if the result would be very accurate. Pelegot and Reiset proved that the percent of butter in the different samples varies according to the length of time the milk has been in the glands. Marchand considers that the best average sample will be got at the evening milking. Fore-milk, is the first of the milking and is very poor in fat, while the strippings are very rich.

Of all the methods that I have tried or seen I prefer

the following. Evaporate about 10 grms. to dryness on a water-bath, and dry at 90° to 100° C. weight = total solids. This takes about three hours. As to the final temperature Vernon and Becquerel say 80°. Hopper and Seyler 120° and others vary. But I have found at 100° the residue is sometimes discolored. Soften with a little alcohol and ether, heat and decant, repeat the washings with hot ether three times, dry as before and the loss of weight equals the fat. The fat is usually too low by this method. The ether solution may be saved and distilled so that there is comparatively little loss. The residue is then macerated for 10 or 15 minutes, repeatedly stirring so as to reduce the whole mass to a homogenous consistency with diluted alcohol and hot water. This dissolves the sugar and the soluble part of the ash. Filter on a weighed filter of known ash. dry the filter at 90°, weigh and ignite - and again weigh. The loss less the difference of the filter gives the casine. Evaporate the filtrate to dryness, weigh

ignite gently, and again weigh, the loss equals the sugar. The residue of soluble ash added to the casine residue of insoluble ash gives the total ash. I have tried evaporating milk to dryness with sand which had previously been washed with HCl and ignited, but I do not see any particular advantage to be derived except that the milk may come to dryness a little quicker. But there is a greater chance for loss and if a complete analysis is to be made it takes longer in the end. Gypsum is also recommended but I do not like that either and if the gypsum be not entirely pure or if there be any anhydrite formed by over heating it would lead to quite serious errors. Plaster of Paris has the fault that two samples must be used in complete analysis. However simply drying alone has its faults as it requires much skill and practice to get the total solids perfectly free from water. $BaSO_4$ has also been suggested for drying.

For the determination of the fat alone

these processes are very convenient and by transforming the dried residue to a continuous extractor apparatus whereby the ether is run through the mixture again and again very accurate and satisfactory results are obtained. The same is true for the sugar, in the extraction of which however some would use alcohol and others water.

As the casein however is pretty soluble in hot water the last would vitiate the results. Of the two, Plaster Paris and Sand I much prefer sand as being "more chemically inert and more porous"

This comparative table gives the results obtained by the greater chemists and in most cases the large varieties are due to different processes employed.

The comparative table spoken of above will be found on the page following ie page 13 –

Chemists	Solids not fats			Casein			Sugar			Fats		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max	Mean.
Wanklyn	8.70	9.99	9.3			8	87			1.22	3.50	3.2
Bell	9.2	11.3	9.9			9.14						3.70
Chevallier												2.75
Felhol & Joly												8.25
Meichaud				1.90	4.37	2.31	4.85	5.53	5.02			3.72
Simon					7.20		2.71					
Smith			9.96			5.5			3.8			3.6
Baumhauer	8.35	9.73	9.	1.13	2.08	7.53	3.91	4.74	4.17	1.97	4.34	2.85
Pierce	8.35	12.46	9.6	3.77	5.55	5.52	3.44	4.54	3.97	2.74	3.88	3.47

As to the determination of the purity of milk from one constituent, it can never be done. However what will come nearest this is probably the best. The Society of Analysts have agreed to call all milk adulterated which contains less than 9 percent of solids not fats or 2.5 percent of fat, but according to their own published tables their poorest "pure milk" will

allow of an adulteration with 2.5 percent of water and the best samples 20 percent. The creamometer which was once in vogue, simply shows the varying amount of cream but as the composition of cream differs so much it has had to be discarded. Another objection to this is that the results of the same milk vary. All the milk used in cities has been subject to more or less agitation. Milk shaken in this way does not give a miform rise of cream as the milk has become more or less lumpy. The results of my own experiments are as follows.

Time shaken.	Proportion of cream	
2 Min.	11	11
5 "	16	16
11 "	14	20

This was after standing four hours and the results are in millemeters instead of percent and are simply relative. The shaking was only moderate. Most of all the instruments invented in connection with milk inspection, which have been recognized

to any great extent is the Lacto-densimeter or Lactometer of France which is simply a delicate Sp. Gr. instrument. Errors equal to 5 percent of adulteration have been noticed, due simply to the amount of milk which adheres to the stem owing to oscillation. As milk is not a homogeneous nor even a constant fluid this will never be of any use The fat globules are lighter than the menstruum and we have a case similar to that which happens when air bubbles are dissolved in a liquid. These attach themselves to the lower end of the instrument and vitiate the results And right here we may say again that city milk always contains some air also, in solution which alone would largely change the result. The following experiment of taking the Sp. Gr. of milk by a common urinometer shows what errors can happen to milk that has been shaken.

Time shaken	2 min.	5	10	15
Sp. Gr.	1.037	1.030	1.032	1.027.

With a delicate

Lactometer greatir differences would be noticed. The Lacto-
butyrometer of Marchand is a cylinder in which a known
amount of milk is mixed with a solution consisting of equal
parts each of Wa 40 ether and alcohol. He claims that
the butter will be dissolved out upon shaking and separate
as the upper layer where it may be read off. With me it
has always failed to do as he claims. There is no separation
in the least, of the butter. With the Galactometer and Lacto-
scope I have had no experience, Last which comes under
my notice and with which I am most familiar is the
Centrifugal machine. See Fig. I It consists of the two
wheels connected by a band of leather so as to increase
the motion given to the larger wheel. To the ~~separator~~ Wheel is
firmly fixed the upright, which on its top bears another
plate. To the periphery of this plate are fastened rings
loosely hung on two pivots so as to allow of an easy mo-
tion to and fro of the tubes of milk, which tubes are

slipped into the rings hanging vertically. When the centrifugal motion is imparted to this plate, the tubes turn on the loose pivots and assume a horizontal position. Fig. II shows a horizontal section of the upper plate and rings. Heavy Bohemian glass test tubes were used in the experiment. They held about 20 c.c., Diameter .015-in Length about 15 c.m.. They were carefully graduated to read percents. The theory of the instrument is, that the centrifugal force imparted to the milk; by the varying Sp. Gr. of the cream and skim milk will separate the two completely. Now cream itself is a mixture of the fats with water, casein and sugar and ash the same as milk, and it is obvious therefore that the longer the centrifugal force continues to act, the more complete will be the separation, and it is necessary that some standard time be taken and also a certain number of revolutions per minute, in order that the results may be uniform.

At first I took five minutes as the time but as the

sharp separation was not always effected by that time the data are of no especial use. I changed to ten minutes and in almost every case the separation was sharp and distinct. Some samples especially those rich in creame must be diluted with equal volumes of water. This aids the separation and distinct reading very much, of samples containing 12 percent or more of creame. Nearly all my work was done at 7000 revolutions per minute. Nothing less did good work and my machine would not stand much more. As to the temperatures I did not succeed in satisfying myself as to what was best, but I liked best about 18°. Much higher than this gave me results at total variance with the creamometer and I had no opportunities of trying much lower, for that was the temperature of the Labratory and though I tried cooling my milk I found in all cases that at the end of ten minutes the milk was the same temperature as the room. To aid the separation I used solutions

of lactic acid of varying strengths but could see no value in it. As compared with milk allowed to stand twenty-four hours, the results are quite uniform and when there has been much variation I have generally noticed that my machine milk was too warm or had been too much shaken so that the milk was lumpy.

Comparative table

No.	5 min.	10 min.	Standing 24 hrs.	Diff	Temp.	Date
1	6.2	6.2	6.6	.4	18°	Feb. 12
2		11.9	10.8	-1.1	18°	" 12
3	18	17.	16.2	-.8	21°	" 27
4	10	8.3	10.	1.7	21°	" 27
5	6.2	6.2	9.2	3.	—	" 27
6		8.4	10.	1.6	19°	" 28
7		3.	4	1.	21°	March 1.
8	14.2	13.3	20.	6.7	21°	" 1
9		6.0	8.4	2.2	21°	" 1

No.	5 min.	10 min.	Standing 24 hrs.	Diff.	Temp.	Date
10		6.2	9.2	3.	27°	March 7
11		11.1	12	9	27°	~~
12		6.7	9.2	2.5	13°	~~
13		3.8	10	6.2	60°	~~
14		9.9	13.3	3.4	18°	Apr. 12
15		3.3	11	7.7	43°	" 11
16		8.3	11	2.7	—	" 11
17		9.9	13.3	34	20°	" 12
18		10.	12	2	20°	" 16
19		9.3	11.1	1.8	—	" 16
20		9.5	11	1.5	18°	" 20
Mean		9.27	10.92	1.71		

This excludes Nos. 8. 13, and 15 which are undoubtedly erroneous, either in samples reading or as I have suggested in being too hot.

The centrifugal machine does give results that compare directly with the creamometer, but there is the same objection that the cream varies in the percentage of fat, and we have not yet solved the problem. Wanklyn gives the variations of fat in cream as between 14 and 44 percent. My own figures are as follows.

Cent. cream of fat	Milk	Standing cream	Milk
16.8	1.42	11.88	1.60
13.4	1.35	26.77	
18.64	1.34	34.98	
11.8	2.27		
30.58	2.10		.56
55.13	2.7		

These analyses of cream and milk were made, first by taking about .5 gm. of cream from the tubes, by means of a platinum spoon, to a stoppered cylinder of about 40 c.c. capacity and 5 c.c. of the skimmed milk to another. The weights

carefully determined, and then treated with a drop or two of NaHO solution, strength 2 to 5 of water and with about 20 c.c. of ether, well shaken and allowed to stand about 2 hours. In the case of creams about 5 c.c of water iwas added also. The ether was then decanted into a weighed beaker and a fresh portion of ether added, well shaken and allowed to stand till separated, decanted and added to the first washing. To avoid heat the ether was evaporated by means of a bellows The fat was then quickly dried, to avoid loss, at 100° cooled in a dessicator and weighed. I do not like this method for I do not belive it is very accurate although I have no comparative data. My next was to take a tube of heavy Bohemian glass draw it down to a fairly small point and trim the edges to fit the rings of the machine. A tube of the same length as the test tube just described, open at both ends and pointed, was fitted into the bottom of the larger tube by means of a piece of rubber as

shown in Fig. III. By carefully raising the small tube the whole of the skim milk may be drawn off and a total and complete separation of the cream and milk made. Plaster of Paris is now added to the separate portions after they have been weighed, the whole mass dried transfered to a continuous ether apparatus and the fats extracted, and dried; and weighed as in the Babcock process.

As has been before said the top layers of cream contain the most fat and as these are the only ones which can be removed by the platinum spoon we do not get a fair average sample of the cream for the Babcock process Though under ordinary circumstances the analysis is much more easily and quickly made by that process.

In order to get rid of the air bubbles and the adhesion to the sides of the tubes which latter is especially annoying also in milk which has been shaken. add a drop of ether and it cleans the sides and

lessens the meniscus immediately. It is often hard to read the creamometer correctly for the same reason, and too I have noticed that the meniscus after standing six hours is greater than the day before especially if the room is warm This is due I suppose to evaporation of the water. If any of this water should come from the milk the reading for cream would be slightly wrong for the cream would settle back into the milk again. But if it comes from the top layers of the cream the reading at the bottom will still be correct

Conclusion

From the work I started to do, which was to test the centrifugal machine as a ready, quick, convenient, and accurate means of testing the purity of milk I have deviated some because I do not believe that the determination of any one constituent will enable me to detect small adulterations. From the tables I have given I think I may safely say that the centrifugal machine is as good means of

testing milk as the creamometer. In this respect it is much better, a larger machine could be easily made that would hold fifty or a hundred tubes each containing different samples of milk and in less than a half hour these could all be tested and as before said the element time is entitled to a great deal of alteration in milk analysis.

The Centrifugal Machine in ten minutes with 1000 revolutions per minute does separate distinctly the cream from the milk: not so perfectly as by allowing the milk to stand twenty-four hours, as is shown by the amount of fat in the centrifugal milk and also by the fact that more cream will rise in the course of a few hours, but perfectly and regularly enough I think if the temperature be kept about 18° to warrant any and the same assumptions that are and can be made of the creamometer.

It is true that neither the creamometer nor the centrifugal machine will detect watered milk and it is

also true that the value of milk as a food does not depend entirely upon the cream but I believe that milk is generally sought for in actual use on account of the amount of cream that will rise.

It is the cream and not the nutritive value of the milk that is generally desired in the cities. It may be well enough for sanitary officers to insist on a certain amount of solids being in the milk used in their institutions but the housewife will generally prefer that the milk raise a certain percent of cream. This is shown by the creamometer or more quickly by the centrifugal machine. From the skim milk the "solids not fat" may be much more quickly obtained as the milk is not covered with such a heavy impervious coating of the fats and casein, and in an institutions where they admit milk that contains a certain percent of "solids not fat," which is perhaps the truest basis of milk analysis, the centrifugal machine may do good service in

removing the greater part of the fat before drying. The percent of required cream would have to be changed. From 8 to 9 percent of cream is thrown up instead of the old 10%. But as experience has shown that in creameries practically a larger percent of butter is obtained by the centrifugal process, than by allowing the milk to stand so we might expect that a different length of time and a greater motion might give us other results that would more nearly coincide with those of the creamometer and I feel assured that with an even temperature the results of the centrifugal machine will be quite satisfactory. My figures show that the composition varies very much so that I can not say that this forms an accurate method of determining adulteration of milk.

~ The End ~.

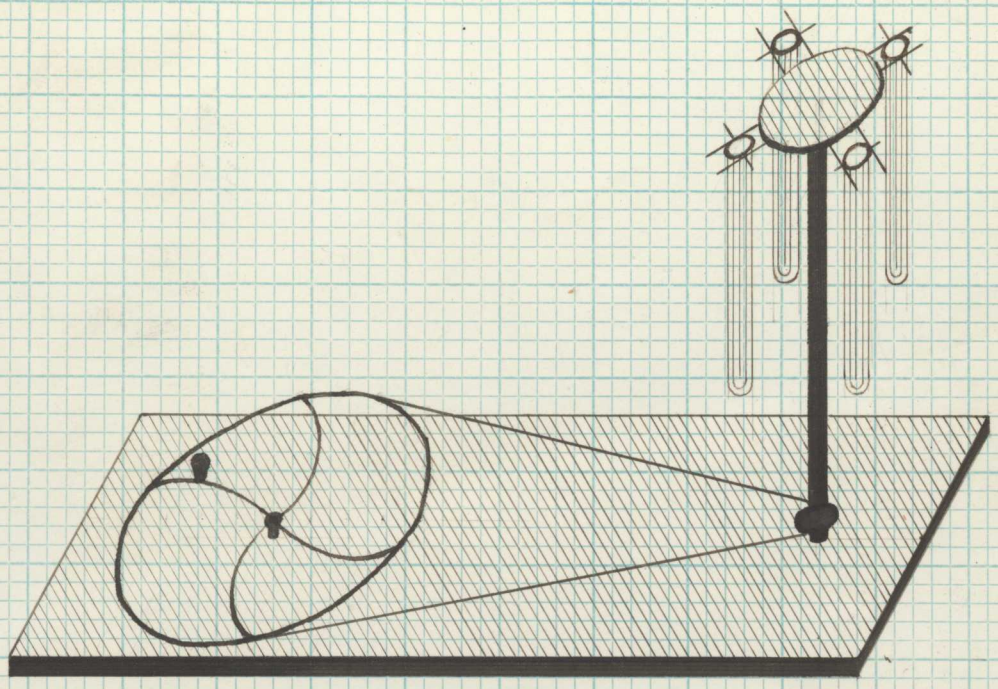

Figure 1.

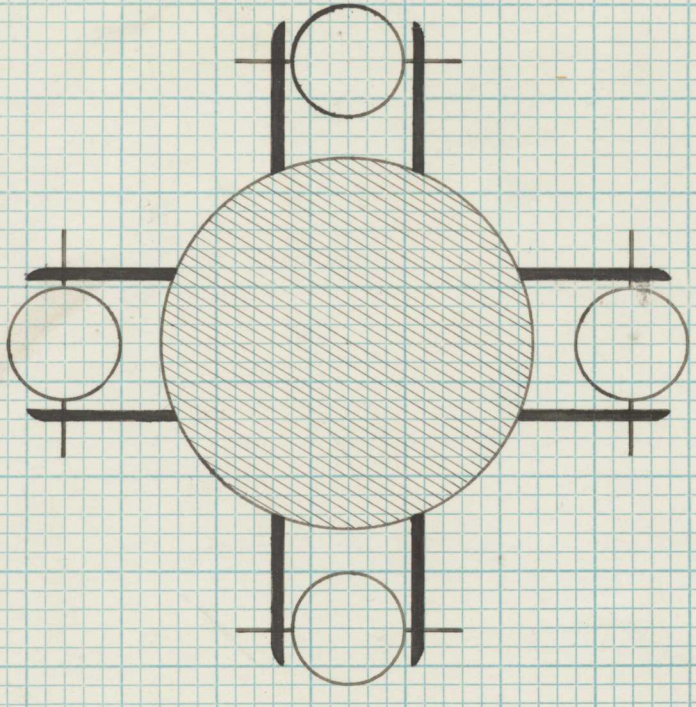

Figure 2.

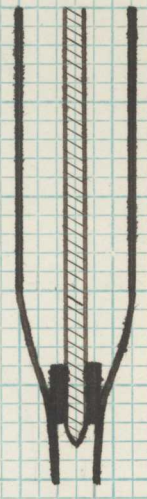

Figure 3.

Thesis:—

"On the Separation and Estimation of Cinchona Alkaloids;"

for the

Degree of D. S.

In the School of Chemistry,

by

Joseph D. Weis.

1883
N2

The most valuable of all vegetable medicines are the Cinchona alkaloids, and especially quinine, obtained from cinchona bark, also known as Peruvian bark, Jesuits bark, china bark, etc. The genus cinchona, from which the bark is obtained, belongs to the Linnean class of Pentandria Digynia and to the natural order of Cinchonaceae. It is originally found only in South America between south latitude 20° and north latitude 10° and chiefly on the eastern slope of the second range of the Cordilleras, but it has since been cultivated in Java, China, Japan, and other eastern and southern asiatic countries. The cinchona is an evergreen tree and is found only in the highest mountain regions.

Some of the species are often of great magnitude

but after the trees have been felled, an after-growth, springing from the roots, appears, the stems of which are often only eight or ten feet high.

The trees are cut and piled by the Indians, who go in large parties. They are cut close to the root from which an after growth, which may after eight or ten years again be cut, springs. The bark is carefully dried and wrapped in woolen cloth and then in hides.

The reputation of this bark as an almost infallible cure to ague and intermittant fever, was known to the native Peruvians, and many stories are told, to account for the discovery of its virtues. In 1638 its medical properties were first made known in Europe, and it was introduced from Peru by the countess Chinchon, wife of the vice-roy of Peru,

and whom it had cured of an intermittant fever, and from whom it received the name of Cinchona. The bark was taken to Rome by the Jesuits and then distributed to all the Jesuit stations through which it could only be procured. Hence the name "Jesuits Bark."

Although it was at first vehemently opposed by all medical men, and the protestants entirely discarded it, it was soon, on account of its many cures, generally introduced, and in 1659 and ounce of the bark sold at five pounds Sterling.

Cultivation has done a great deal to improve the bark, and to increase the relative amount of alkalids. The amount of total Alkaloids varies from 1½ per cent to 10 and 11 percent; and the amount of quinine from ½ to 8 percent. The amount of total

alkaloids in the bark of the uncultivated tree seldom exceeds 6 or 7 percent, while in the bark of the cultivated tree it is increased to 10, 11 and even 12 percent.

The cinchona barks are divided into the "true" and the "false". The "true cinchona barks" are again divided into the "grey", "yellow" and "red".

The "grey cinchona barks" (cinchona fusca) are obtained from the branches of five different species, namely:- Huanco, Loxa, Pseudoloxa, Huamalies and the Jaen bark.

The yellow bark (cinchona flava) consists wholly of the base or inner bark, and is obtained from the larger branches and stems of the Calisaya, royal or king's tree.

The red barks (cinchona rubra) are from the species - cinchona rubra rubrosa, C. succirubra, and

C. rubra dura.

The false cinchona barks are obtained chiefly from the genera – Ladenbergia and Exostemma. They contain no cinchona alkaloids, and when pulverised and heated in a test-tube they yield nothing but a dirty yellow or a brown tar.

The principal alkaloids found in true cinchona barks are the following:– Quinine, Cinchonine, Quinidine, Cinchonidine and Amorphous alkaloid, together some of a lesser importance, such as Quinamine, Quinacine, etc.

Quinine, or the Cinchona resin of the older chemists, is by far the most important of the cinchona alkaloids, on account of its tonic and antifebrile properties. It has the formula $C^{20}H^{24}N^2O^2$. It was first obtained in an impure state by Gomez

of Lisbon and by Pfaff in 1811. In 1820 Pelletier and Caventou separated it from the other alkaloids, and in 1838 Liebig determined its composition. The formula for the Hydrochlorate, which may be taken as a representative of all salts of quinine, is - $C^{20}H^{24}N^2O^2 . HCl$.

Cinchonine, the formula for which is $C^{20}H^{24}N^2O^2$, was discovered by Gomez of Lisbon in 1811, but its true nature was established by Pelletier and Caventou in 1820. The formula for the Hydrochlorate is $C^{20}H^{24}N^2O^2 . 2HCl$.

Luinidine ($C^{20}H^{24}N^2O^2$) is isomeric with quinine. It was first observed in the quinoidine of commerce by Henry and Delondre, in 1833. Van Heijningen soon after separated it from the other alkaloids and determined its composition.

Cinchonidine ($C^{20}H^{24}N^2O^2$) is isomeric

with cinchonine. It was discovered by Winkler. The formula for the Hydrochlorate is $C_{20}H_{24}N_2O_2$. HCl.

The methods employed for the estimation of the total alkaloids in the barks and for their separation are quite numerous. The older methods, among which that of Hager may be taken as a representative and which has proved to be very efficient, consisted in dissolving the alkaloids in an acid, and precipitating them with lime or carbonate of Soda. The later methods consist in extracting the alkaloids with alcohol or ether, precipitating with caustic Soda, and dissolving in chloroform, together with some purifications by means of Calcium Hydrate. It has been my object to test these different methods and to see which are the most efficient. The methods of separating the different alkaloids, are based

upon the difference in solubility of the alkaloids and their salts.

The bark employed in these experiments was that of the Calisaya. The methods employed for the estimation of total alkaloids were those of Hager, H. Meyer, Prollius, Kissel, J. E. de Vrij and Biel.

Hager's Method. - Digest 10 grams of bark for a short time with 130 grms. water, and 10 grams of Potash ley with a specific gravity of 1.35. Then add 15 grams of sulphuric acid (sp. gr. 1.115) and boil for 20 minutes. When cool, add water to make up to 100 c.c. and filter. Precipitate the measured filtrate with 50 c.c. of Picric acid solution, saturated in the cold. Filter, dry at 100°C. weigh and estimate. As will be seen from the table below,

this method although quite old, proves to be quite efficient, giving nearly the same result twice.

Mayer's Method.- (Journal of the Chemical Society, March 1883. p. 388.) Place 10 grams of the finely powdered bark, 12 grams freshly prepared calcium hydroxide, with 180 c.c. of 90 per cent alcohol into a tared flask. Boil on water bath for one hour. When perfectly cool, make up the solution to 190 c.c. with 90 per cent alcohol. Shake up carefully, allow to settle and filter off 100 c.c. This amount represents the alkaloids in five grams of bark. Now place the solution in a dish previously rinsed with alcohol; add 20 c.c. of one per cent diluted Sulphuric acid. Warm on a water bath, and evaporate the alcohol with constant stirring. The quinovine, quinoic acid and waxy fat remain suspended in the

fluid, which now amounts to about 10 c.c. When cool, add 10 c.c. water, and filter into a separating funnel of 150 c.c. capacity. Repeatedly wash the dish and filter with distilled water, until the filtrate gives no longer a precipitate with Picric acid. Then add 50 c.c. Chloroform and caustic Soda to strong alkaline reaction. Shake up well; allow the liquid to clear, and run the chloroform off into a tared flask, and remove it by distillation. The flask is now heated in an air-bath for one hour at 100° C. Cool in an exiccator and weigh. The operation with Chloroform is repeated as long as a weighable residue is found. Three times will usually suffice.

Bolling Method. — (Fresenius Zeitschrift, 1st Heft, 1883, p. 132). Macerate ~~three~~ five grams of the dry powdered bark with a mixture of 38 parts alcohol

10 parts chloroform, and two parts of ammonia. After allowing it to stand for some hours, pour off as much of the clear liquid as possible, and mix it with 5 parts of calcium hydroxide. This will decolorize the solution. Filter and evaporate the solution in a tared dish. Dry at 100°C. and weigh. Weight equals that of total alkaloids.

Kissel's Method.- (Pharm. Zeit. 1st Heft 1883 p. 132). Macerate three grams of the dry powdered bark with 30 grams of the following solution:- 88 parts ether, 4 parts ammonia, and 8 pts. alcohol, for two hours. Evaporate 20 grams of the solution, take up the residue with some very dilute sulphuric acid and hot water. Filter, and the waxy fat and quinic acid will remain on the filter. When the filtrate has cooled, precip-

itate the alkaloids with caustic Soda Solution. Filter on a tared filter, dry at 115°C. and weigh. From this the total alkaloids are readily estimated.

The great trouble with the last, as with Rolline's method, is that the amount of bark used is too little.

J. E. de Vrij's method.—(Fresenius Zeitschrift, 1st Sept 1883, p. 132). Vrij modifies Kissel's method by mixing 10 grams finely powdered bark with 200 grams of Kissel's solution, known as Rolline's Ether extracting Solution, and macerating with occasional shaking. Pour off a weighable portion of the clear liquid, distill off the ether and wash the residue into a tared dish with alcohol. Evaporate on a water bath. To purify the alkaloids, dissolve the residue in dilute Hydro-chloric acid, filter

and thoroughly wash until filtrate no longer gives a precipitate with caustic Soda. Shake up the solution with Caustic Soda and chloroform, and allow to stand for 12 hours. Separate off the chloroform by means of a separating funnel. Distill the chloroform, dissolve the residue in alcohol, evaporate in a tared dish and weigh.

Biel's Method.—(Pharmaceutical Jrnl. 1st Dec. 1883, p. 482). Macerate 20 grams of the dry powdered bark with 200 grams of Rolling's Ether extracting solution, in a stoppered bottle for 4 hours. Filter rapidly and decolor the solution with calcium hypochloride. Evaporate 100 grams of the filtered solution on a water bath. Dissolve the residue in a few drops of Sulphuric acid and hot water. Cool, filter and wash. The filtrate is rendered alkaline with

ammonia and thoroughly shaken up with 20 c.c. chloroform. The chloroform is separated out by means of a separating funnel and distilled. This chloroform separation is repeated 3 or 4 times. The residue is dried at 110° C and weighed. In order to get more accurate results, the residue may be dissolved in acetic acid (diluted). Filter on a sand filter. Dry at 110° C and weigh the residue as resin which is subtracted from the original weight.

This last method and that of Vrij have to me proven to be the most accurate.

The method employed for the separation and estimation of the several alkaloids, was that of J. E. de Vrij. Vrij's method is based upon the following facts:—

1. great solubility of gummi and amorphous

alkaloid in Ether, and the very slight solubility in that liquid of quinidine, cinchonidine and cinchonine.

2.- Great solubility of Iodosulphate of amorphous alkaloid in alcohol, and the very slight solubility in the same liquid of Iodosulphate of quinine.

3.- Difference between the solubilities in water of tartrate of cinchonidine, and the tartrates of cinchonine and quinidine.

4.- Difference between the solubilities in water and alcohol of the hydriodide of quinidine and the hydriodide of cinchonine.

The method is conducted as follows:—
The total alkaloids of which the weight is known, are digested with 10 to 20 parts of Ether and filtered. The alkaloids are now separated into two groups:—

(a). Part soluble in Ether. - Evaporate the Ether and dissolve the residue in ten parts of proof spirit, with 1/10 of Sulphuric acid. Add alcoholic Iodine, until a precipitate is formed no longer. The quinine is thus precipitated as Herapathite. Filter, wash and dry at 100°C. One part of Herapathite represents .565 parts of pure quinine. The amorphous alkaloid may now be estimated by difference, or it may be precipitated with an alcoholic solution of Sulphurous acid. Neutralise with caustic Soda, heat on a water bath to expell alcohol, and add an excess of Caustic Soda. The precipitate consists of Amorphous Alkaloid. Filter, dry at 100°C and weigh.

(b) Part insoluble in Ether. - The insoluble alkaloids are dissolved in 40 parts of hot water with a little dilute Sulphuric acid. Add

a solution of Rochelle salts, and allow to stand for 12 hours. Cinchonidine will separate out as the tartrate. Collect, dry at 100°C and weigh the precipitate. One part of the tartrate represents .804 parts of cinchonidine.

The filtrate is mixed with a solution of Potassium Iodide, which precipitates the quinidine. Filter, dry at 100°C. and weigh. One part of the hydriodide represents .718 parts of quinidine.

Cinchonine, which is now the only alkaloid left in solution is precipitated with caustic Soda.

Below is given a Table of the per cent of total Alkaloids as well as of the several Alkaloids in Calisaya bark, as estimated according to the different methods.

Methods.	Total alkaloids.		Quinine.		Cinchonine.		Quinidine.		Cinchonidine.		Amorphous Alkaloid	
Hager.	4.00	3.73										
Mayer.	3.20	3.41	.63	.59	1.13	1.27	.22	.17	1.26	1.15	trace	trace
Rolline.	5.60	5.33	1.17	1.31	1.26	1.13	.35	.29	1.37	1.41	.21	.17
Kissel.	4.91	4.69	1.25	1.37	1.13	1.11	trace	trace	1.24	1.31	.31	.23
Vrij.	4.25	4.01	1.57	1.47	1.02	1.09	.10	trace	1.07	1.10	.48	.49
Biel.	3.97	4.10	1.61	1.49	.98	1.08	trace	.13	1.01	1.00	.40	.38

By adding the several alkaloids as found by analysis, we get the following for total alkaloids:-

Mayer.	3.24	3.18
Rolline.	4.36	4.33
Kissel,	3.93	4.02
Vrij.	4.24	4.15
Biel.	4.00	4.07

Looking at the above table we will see at a glance that Vrij and Biel's methods are the most efficient. The Duplicates in each nearly correspond, as well as do the results of the one method with those of the other.

Meyer's method fails to extract all of the quinine and amorphous alkaloid, these two being not so readily soluble in alcohol.

Prollius and Kissel's methods fail to remove all impurities from the total alkaloids. Hence the difference in the table between the total alkaloids as estimated directly and as estimated by adding the several alkaloids.

Another method for the separation of the several alkaloids, which however I have not used, is as follows:—

The alcoholic solution of the alkaloids just after extraction from the bark, is divided into three or four equal parts.

Evaporate the first part to estimate the total alkaloids.

Evaporate the second part and treat the residue with ether and estimate quinine in the same manner as in Vrij's method.

The third is titrated with Sulphuric acid. Let x = the sum of quinine and quinidine. The amount of cinchonine plus cinchonidine equals alkaloids $- x$; and the quantity of Sulphuric acid used, S, will be given by the formula:—

$$S = \frac{98}{648}x + \frac{98}{616}(\text{total alkaloids} - x),$$

whence x is determined. Subtract from this

the amount of quinine as found above, and we have the amount of quinidine.

The portion insoluble in ether is now dissolved in sulphuric acid and the solution rendered slightly alkaline by adding caustic Soda. Then add Rochelle salts, which will precipitate the cinchonidine as tartrate.

Cinchonine is estimated by difference. This method is but slightly different from that of Vrij; the principles are the same in both.

Below is annexed a table of solubilities of the most important Cinchona Alkaloids, taken from Prescott & Provisional Organic analysis.

Alkaloids.	Water.	Fixed alkalis with H_2O.	NH_4HO with H_2O	Ether.
Cinchonine.	Sol in 2500 pts. boil.	Insoluble.	Insoluble.	Sol. in 400 pts.
Cinchonidine	Sol in 2000 pts. cold.	———	———	Sol in 150 pts.
Quinine.	Sol in 1800 parts.	Insoluble	Soluble.	Soluble.
Quinidine.	Sol in 750 parts.	———	———	Sol. in 20 pts.

Chloroform	Benzole	Chloroform with Acid.	Benzole with Acid.	Amylalcohol with Acid.
Soluble in 20 pts.	Soluble	———	———	———
———	———	———	———	———
Soluble in 50 pts.	Soluble.	Insoluble.	Insoluble	Insoluble.
Soluble	Soluble	Insoluble	Insoluble	Insoluble.