EXPERIMENTS ON MILK ANALYSIS.

By E. Waller, Ph. D.

Legislation regarding food adulteration has been frequent since mediæval times. The most decided step of general importance in English speaking countries however was the act of the English Parliament in 1872, under which, I believe, for the first time, the office of Public Analyst was created. That act has been modified by more recent ones, but the important and essential features have been retained. With the establishment of the office of Public Analyst the formation of a society of Public Analysts for mutual benefit and information naturally followed in the Autumn of 1874 (*Chemical News*, **31**, 58).

The Society at its very inception came to an agreement as to the standards or percentages of the component parts of several of the commonest articles of food, among them milk. The limits were set in the following terms, (*loc. cit.*), "milk shall contain not less than 9.0 per cent., by weight, of solids not fat, and not less than 2.5 per cent. butter fat."

The method of analysis was not, so far as I am aware, officially described, but apparently by a general, informal understanding, the method described by Wanklyn in his book on Milk Analysis (London, 1871) was generally adopted with the exception that the portion taken for analysis (usually 5 grms.) was weighed instead of measured as he recommends. The method consisted in drying this quantity of milk in a weighed dish over the water-bath for 3 hours, weighing to determine the water by loss, and then extracting the milk solids three times with boiling ether, decanting through a filter, evaporating the ether and weighing the fat. Previous to the formation of the Society of Public Analysts I had experimented on Wanklyn's method and had adopted a modification of it, known on this side of the water as the Wanklyn-Waller

method. The modifications consisted in finishing the drying in an air bath at 100° to constant weight, in increasing the number of applications of ether to six or more, and in checking the determination of butter fat by drying and weighing the exhausted milk solids. The filtration of the ethereal solution of fat was also dispensed with as unnecessary, (*Analyst* **9**, *69, and Cairn's Quant. Analysis, New Edition, p. 204*). About the beginning of 1884 the British Society of Public Analysts appointed a committee to investigate methods of milk analysis, and to report a method which should be recommended by the society. While this committee was still occupied with its labors, Mr. M. W. Adams brought up his method, which (*Chemical News,* **10**, *46*) consisted in absorbing the 5 grms., of milk taken for analysis into a paper coil, drying off and determining the water by loss, and then extracting the fat from the coil by means of an extractor of the Soxhlet pattern. This method gives a higher figure for the fat, and after trial was recommended by the committee as the method for fat.

But one additional point in the procedure has been adopted since that time, viz., the previous extraction of the coil with alcohol containing 10 per cent. of glacial acetic acid, followed by an ether extraction, to prepare it for use. The "limits" previously set by the society were also modified in consequence, that for solids not fat being put at 8.5 and for butter fat at 3 per cent., practically an assertion that by the Adams method 0.5 per cent. additional fat was obtained.

In the meantime, in the United States, the National Board of Trade offered in 1880 a prize for an essay on food adulteration, to be accompanied by the draft of an act to be recommended to our legislators. The prize was given to G. W. Wigner, a prominent member of the British Society of Public Analysts, and legislation followed.

Also, in 1884, the New York legislature passed an act creating a State Dairy Commissioner, and legal limits were fixed for the composition of milk which should be merchantable.

The act declared that whole milk should contain at least 12 per cent. of solids, of which 3 per cent. should be fat. Some other States have also passed similar laws. The New Jersey standard

was, I believe, the same as that in New York; that of Massachusetts called for 13 per cent. of solids.

In consequence, the question has often been put in court, not "Is it your opinion, as an expert, that the milk in question was skimmed?" but, "Did this milk contain less than 3 per cent. of butter fat?"—a distinction in which there was sometimes a great difference. When a case was brought into court in which between 2.5 and 3 per cent. of fat had been found by the Wanklyn-Waller method, the counsel for the defense has claimed that since, by the Adams method 0.5 per cent. of fat more was attainable, his client must therefore be regarded as innocent. A logical consequence of this reasoning, would, however be, that as the law had been framed in the light of results obtained by the W-W. method, that 3.5 per cent. of fat should be demanded, and that his client, on the face of the facts, could not be regarded as innocent.

The correctness of the results by the Adams method, is, however doubted by many chemists.*

Some investigations by the writer as to the comparative value of results by the two methods mentioned may therefore be of interest. The milks examined are designated as follows:

	Per cent. water.	Per cent. ash.
A. Certified as genuine	87.29	0.769
B. " "	88.03	0.723
C. " "	86.49	0.763
D. " "	86.94	0.797
E. Suspected sample	87.23	0.698
F. " "	88.86	0.695
G. " "	87.47	0.745
H. Purchased for experiment	87.596	
I. Sample H diluted with two volumes of water	95.963	
J. Cream from H	77.866	
K. Skim from H	88.668	

E. and F. were analyzed by other chemists in New York, in the regular course of business, and not as checks on this work. The

* Wanklyn, *Chem. News*, **53**, *70*.
Review of "*Wanklyn's Milk Analysis*," new edition. *Chem. News*. **53**, *213*, Johnstone. *Chem. News* **60**, *233 and 257*

gentlemen, however, kindly permit me to quote their results (marked X.)

As a part of the investigation, the influence of the size and shape of the dishes used in the W-W. method was tested; the respective merits of the round (hemispherical) bottomed dishes, (recommended by Wanklyn) and of flat bottomed dishes was noted. The classes of dishes used were:

Designation.	Capacity.	Bottom.	Approximate diam. of
O		Flat	$2\frac{3}{8}$ inches
L		"	$2\frac{1}{2}$ "
S	$\frac{1}{2}$ oz.	Rounded	$1\frac{1}{4}$ "
V	1 oz.	"	$1\frac{1}{2}$ "
R	2 oz.	"	2 "
M	6 oz.	"	$2\frac{1}{4}$ "

Some of these dishes, when containing milk solids, were subjected to continuous extraction (marked "Con" in the Table). Mr. E. W. Martin, of the New York City Health Department, has devised a form of extractor which can be used with a platinum dish which I have here for inspection. It consists simply of a flask with a chamber above of sufficient size to accommodate the dish, which is surmounted by a worm condenser, the whole of glass, put together with ground joints. In the side of the dish is a hole through which can be passed a glass tube, suitably bent to form a syphon, by which means the intermittent flow of the ether, common to all extractors of the Soxhlet pattern is secured.

This apparatus was used where in the Table dish "O" with continuous extraction is specified.

Mr. Martin also suggested the use of tinned lead capsules (such as are used for making bottle caps) in which to evaporate the milk solids. When it may be desired to subject them to continuous extraction, they may be pinched together and inserted in the tube of an ordinary extractor. Such dishes are designated in the Table as "L."

No.	Method.	Dish.	FAT. One Extraction. Direct.	One Extraction. By Loss.	Two Extractions. Direct.	Two Extractions. By Loss.	Three Extractions. Direct.	Three Extractions. By Loss.
A	W-W. *con.*	O	3.972	3.740	4.081	3.987	4.091	3.942
"	W-W.	S	3.628	3.531	3.721	3.727	3.746	3.748
"	"	R	3.854	3.741		3.798		
"	Adams.		4.249		4.267			
"	"		4.339		4.563			
"	"		†4.124					
B	W-W.	S	2.957	2.900	3.073	2.963	3.089	3.098
"	"	V	2.977	2.905	3.049	2.969	3.081	3.054
"	"	M	3.237	3.100	3.265	3.041		
"	Adams.		3.277		3.283			
"	"		3.407					
C	W-W. *con.*	O	4.244	4.111	4.284	4.131		
"	W-W.		3.996	3.833	4.244	4.061	4.274	4.153
"	Adams.		4.449		4.467			
"	"		4.458		4.518			
D	W-W. *con.*	L	4.215	3.748				
"	W-W. *con.*†	L	4.000	4.319				
"	W-W.	V	3.728	3.748	3.907	3.907	3.967	3.957
"	W-W.	M	3.686	3.686	3.855	3.825	3.894	3.866
"	Adams.		4.184					
"	"		4.258					
E	W-W.	V	4.721	4.572	4.885			
"	W-W. *con.*	O	5.133	4.780				
"	Adams.		4.960		4.989			
"	"		5.015		5.025			
"	"		†5.151					
"	"		†4.994					
"	" X		5.039					
F	W-W.	R	2.800	2.700	2.897	2.827		
"	"		2.817	2.837	2.877	2.966		
"	W-W. *con.* X	O	3.070	3.080				
"	Adams. X		3.320					
G	W-W.	L	3.146	3.221	3.288	3.416		
	"	L	3.057	3.028	3.315	3.340		
	Adams.		3.823		3.823			
	"		3.812		3.822			
H	W-W.	L	3.439	3.334	3.497	3.641		
	Adams.		3.858					
I	W-W.	L	1.188	1.179	1.228	1.218		
	Adams.		1.450					
J	W-W.	L	14.561	14.592	14.636	14.679		
	Adams.		14.511					
K	W-W.	L	2.358	2.352	2.424	2.468		
	Adams.		2.731					

*Extracted through bone black.

† Treated as prescribed by Wanklyn. Water driven off by drying for three hours on the water bath only.

Samples H to K inclusive, were used for another test upon the relative action of the two methods, as will be detailed later.

In the table by an "extraction" is meant the ordinary degree of extraction usually given in the respective methods, viz., five or six applications of ether in the W-W. method, except when marked "*con.*" In these cases and with the Adams method twelve or more syphonings of the ether occurred. It will be seen that the W-W. method, as a rule, gives results 0.2 to 0.3 per cent. less than the Adams method, and not 0.5 per cent.

CHARACTER OF THE FAT.

The difference in odor between the fat obtained by the two methods was noted by many when the method was first published and tried. No other differences have, however, been pointed out. Tests were made on several of the lots of fat extracted. Though incomplete, the indication seems to be that some material not fat is extracted with the last portions of the fat. Whether it is extracted in sufficient quantity to affect the weight to the extent of difference noted between the two processes, cannot be confidently asserted.

One way of testing was as follows: To the fat from 5 grms. of milk was added about 25 c.c. of hot water and a drop or two of ammonia. After warming for about 15 minutes this was filtered, and the filtrate evaporated until the alkalinity was quite faint. It was then filtered, cooled, and tested with a drop of nitric acid, and one or two of strong mercuric nitrate solution, or with potassium ferrocyanide acidified with acetic acid. The fat obtained by the Adams method usually showed precipitates in this case, which suggested the presence of albuminoids, while that from the W.-W. method showed little or no precipitate of the kind, as the degree of extraction was less. Modifications of this treatment such as more or less dilution, more or less ammonia, or more or less heating, gave phenomena other than those described, but with a uniform method of treatment, the fat from the two methods showed different reactions. Fat extracted by the Adams method through bone black (previously exhausted with ether) gave reactions the same in kind, but apparently differing in degree from that obtained in the ordinary way. The difference in proportion (percentage) was practically *nil*.

Attempts were made to determine nitrogen in some of the fats,

but it was apparent that some disturbing influence existed, for which the most rational explanation seemed to be that the paper of the coils had absorbed small amounts of ammonia salts.

Extractions by either method could not be carried to the point where no visible residue was left on evaporating the ether, although it soon reached a point where it was unweighable. That from the W-W. method was fatty in character and appearance, although there appeared usually to be something else with it. That from the Adams method was less uniform in appearance, a portion resembling fat to the eye, but it was sticky to the touch like a soft resin. It was perfectly soluble in ether, and insoluble in water. When a drop of ammonia was added to the water, a partial solution seemed to take place, and it detached itself in white scales, which turned brown with strong ammonia, becoming green on exposure to the air. The weak ammonia solution on boiling up with Fehling solution and standing overnight showed a slight red deposit.

I have made an extractor, put together with ground glass joints throughout, to test thoroughly the question as to whether anything was due to the corks used in the connections. I have as yet had no opportunity to try it.

Dilution and Skimming Tests.

Samples H *to* K *inclusive.*

To test these methods in another way, some milk was purchased and the specific gravity taken. This was found to be 1.0325. A portion was diluted with exactly two measures of distilled water, and another (weighed) portion was skimmed, and then whole milk, dilution, cream and skim subjected to analysis by both processes. The results have already been given, but for convenience they may be restated.

Dilution.

Milk.	Method.	Per cent. Water.	Per cent. Fat.	
			Direct.	By Loss.
Whole milk H	W-W	87.596	3.497	3.641
" "	Adams	"	3.858	
Dilution I	W-W	95.963	1.228	1.218
"	Adams	"	1.450	
Calculated by	W-W	95.777	1.190	
"	Adams	"	1.313	

It seemed more than probable that the apparent percentage of fat by loss on H was partially due to water unexpelled in the first drying (3.631—3.497=0.144%). A correction and recalculation on this assumption will of course show no change as to fat, but gives figures for water and solids not fat, more in accord. This would give :

	WATER.	SOLIDS NOT FAT.	
		By WW.	By Adams.
Whole milk H	87.740	8.763	8.402
Dilution I	95.963	2.809	2.587
Calculation for I	95.826	2.910	2.861

The fat in both cases is a little higher than the calculation calls for. In the case of the W.-W. method by 0.038, in the case of the Adams method by 0.137, a result more favorable to the consistency of the first named.

Skimming.

A small stoppered globe with a stopcock below, was weighed first empty and then, after partially filling it with the (well mixed) milk. After allowing it to stand quietly for about three hours, it was again weighed, and then the skim was run off from below, and the globe and cream weighed. The cream was run off into a separate beaker, and after pouring a few times through the globe to insure mixing, the products were analyzed as before. The data obtained were :

Weight of original milk	105.0065
After standing three hours	105.0020
Weight of cream	9.4330
Weight of skim	95.5690

Analysis :

	WATER.		FAT (W-W)		FAT (Adams).
	Actual Result.	Corrected.	Direct.	Loss.	
Cream	77.866	77.909	14.636	14.679	14.516
Skim	88.668	88.712	2.424	2.468	2.731

Calculation :

		By W-W.		By Adams.	
	Grms. Sold's.	Grms. Fat.	Grms. S. n. f.	Grms. Fat.	Grms. S. n. f
In 9.433 grms.					
Cream	2.0879	1.3086	0.7073	1.3688	0.7191
In 95.569 grms.					
Skim	10.8299	2.3166	8.5133	2.6100	8.2199
Sum	12.9178	3.6972	9.2206	3.9788	8.9890
In 105.002 gr's.					
Whole milk	13.0224	3.6719	9.3525	4.0510	8.9734
CORRECTED FOR WATER, AS BEFORE.					
In 9.443 grms.					
Cream	2.0838	1.3086	0.7032	1.3688	0.7150
In 95.596 grms.					
Skim	10.7878	2.3166	8.4712	2.6100	8.1778
Sum	12.8716	3.6972	9.1744	3.9788	8.8928
In 105.002 gr's.					
Whole	12.8732	3.6719	9.2013	4.0510	8.8222

Result by W-W. 0.0253 grms. high.
Result by Adams, 0.0722 grms. low.

With the cream the Adams method gives lower results than the W-W. method. This was evidently due to the selective action of the paper of the coil, it having been found exceedingly difficult to cause it to absorb the cream. The aqueous portion was more readily absorbed.

The W-W. method, though a little higher than calculation as before, gives closer results than the Adams, although by making an allowance for the imperfect absorption of the cream by the coil in the latter case, it would appear that each process is fairly consistent with itself, a result which was not anticipated. It suggests the query whether milk does not contain some constituent slightly or not at all affected when the milk solids are dried in a dish, which is however rendered soluble when the milk is dried on a coil of paper. Wanklyn has suggested a probable change in the albuminoids of the milk induced by drying on the coil.

The conclusions from these experiments may be thus summarized :

1. The determination of butter fat in milk by the Adams, method, does not usually give results 0.5 per cent. higher than by the W-W. method.

2. In the W-W. method more satisfactory results are obtained by extracting a few times with ether, drying and then extracting again, than by extracting without drying between.

3. In either case the extraction of the last portions of the fat appears to remove something not fat. More of this material appears to be remove in the case of the Adams than of the W-W. process. This material may exist ready formed in the milk, or may be formed by the physical treatment to which the sample is subjected.

4. In the W-W. method, flat bottomed dishes 2 to 2½ inches in diameter give the most satisfactory results.

5. With fairly rich cream, dilution or some similar device is advisable when the Adams' method is used.

6. The determinations of water in milk, are liable to be too low rather than too high.

Finally, attention should be called to the fact that the assertion that milk contains water, butter fat, casein, lactose and salts is only a broad general statement, which leaves out of account the true complexity of substances which undoubtedly exist in that fluid.

SOME CHEMICAL PRODUCTS OF BACTERIAL GROWTH, AND THEIR PHYSIOLOGICAL EFFECT.

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For a short time I have been engaged in studying the chemical products formed by the hog cholera and swine plague germs by their growth in artificial culture media, and have succeeded in isolating an albumose and ptomaine from each.

To distinguish the products the names *Sucholotoxin* and *Sucholoalbumin* have been given to the substances from the hog cholera cultures, and *Suplagatoxin* and *Suplagoalbumin*, respectively, from the swine plague cultures.