

T H E S I S.

METHODS OF ANALYSIS USED IN U. S. GOVERNMENT NUTRITION
INVESTIGATION AT THE UNIVERSITY OF TENNESSEE.

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By

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For

The Degree

OF

Master of Science.

(Under Dr. Chas. E. Wait.)

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Methods and Results of Methods used in the Nutrition Investigation of U.S. Government at University of Tennessee.

The following methods and results represent the manner in which the analyses of the nutrition investigation have been carried on in the University laboratories for several years, and also many additional improvements of standard methods different from other laboratories. In classifying the analyses the four main heads which are discussed are:

- (1) Determination of Ash
- (2) Determination of Nitrogen
- (3) Determination of Water and Fat
- (4) Determination of Heat of Combustion.

In making the determination of each constituent the following order will be taken throughout each determination, being thus grouped to make a more systematic arrangement:

- (1) Preparation of Sample and amount taken for analysis,
- (2) Reagents used in making determination,
- (3) Apparatus,
- (4) General remarks and peculiarities,

Also in taking the samples of analysis which have been made the past year, the following grouping will be followed:

1. Legums (Whippoorwill Peas) No Sample.....(585)
2. Bread.....(586)
3. Salt Pork.....(605)
4. Feces.....(607)
5. Butter.....(621)

6.Milk.....	(603)
7.Urine.....	(591 ^a)

Determination of Ash.

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PREPARATION OF SAMPLE (a) LEGUMES. A fairly large amount of the peas, say 300 grams, are taken at the beginning of the experiment from the main bulk, and ground into very fine particles, this being affected by placing in a coffee mill and grinding through several times, then sampled in the usual way by dividing into quarters and taking opposites. It is then transferred to a smaller bottle and preserved for use in the other determinations.

(b). Bread. In the experiments of the year 1901-1902, which continued for twelve days each day 80 grams of bread would be taken from each loaf in different parts so as to insure a good sample, placed in oven for 24 hours and thoroughly dried at a temperature of about 100° c.. After its drying was completed its weight was then taken, and the bread now ground in the same manner as the legumes.

(c) Salt Pork. In the preparation of this sample greatest care is taken to get the fat thoroughly mixed. About 100 grams are taken from the main part of the bacon, placed in a saucer, heated in the oven for 24 hours and then transferred to a large porcelain mortar, and ground until all the particles have been thoroughly mixed, then transferred to a bottle and used throughout all the other analyses.

(d). Feces. The entire deposit of feces on tin is placed in a special oven and heated until thoroughly dry, weighed and transferred

ferred to large porcelain mortars and the large lumps broken up. Then ground in a mill and sifted in a specially designed arrangement consisting of a can with a sieve in the middle and with a tin top fitting in as in the case of a bucket. The contents from the mill are poured on the sieve and shaken well. That which does not go through is taken out and ground more and the operation is continued until all has passed through.

(e). Butter. In each experiment several pounds of butter are used, each pound being sampled separately and the whole mixed. A special sampler is used, consisting of a tin apparatus shaped in the form of a half moon, tapering to a point with a cross piece at the other end. This is bored into several different parts of the butter the whole placed in a mortar and then transferred to a glass-stoppered bottle, and thus when a sample is to be taken out the whole is melted and shaken well before so doing.

(f). Milk. Fifty cubic centimeters were taken each day from the main bulk, placed in a large flask, thoroughly mixed and sample taken from this.

(g). Urine. The preparation of urine sample was not necessary the determinations of the nitrogen being made and the whole bulk used as sample, except in preparing the sample for the calorimeter, composites every four days were made by mixing the four day urine and taking out about five hundred c.c for sample. In the sample for the calorimeter urine blocks were used, also nickel capsules, the weight of both being obtained and then the block saturated twice with urine. The following represents a block sample prepared;

No.)	No.)	Weight)	Cap+	)Calrs)	C+B+	)	)C+B/R.)	)Total	)Heat
Cap(Ur.)	(Cap.	(Block	(Block)	Urine(Urine	(C+B+U.)	(Urine	(Urine	(Urine	(Comb.
160)	626)	2.2934)	2.9314)	2763	5.2386)	2.3054)	5.4062)	2.3988)	4.7042)
(	(	(	(	6380	(	(	3.0074(	(	(

Estimation of % of Ash in Sample.

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In estimation of the per cent of ash in the samples duplicates are always made. A sample of two grams is weighed into a weighed Platinum dish a low heat is then applied until the sample after having taken fire ceases to burn. The dish is then allowed to cool, placed on a white glazed paper and the contents should now be ground to a fine powder with an agate pessle. To prevent any of the contents of the dish from being lost while grinding, a large filter paper with an aperture in the middle is placed over the top of the dish, and through this opening the pessle is placed and the contents ground. After being finely pulverized about 10 cc. of boiling water is added so as to dissolve all the NaCl and other soluble salts, which are contained in many of the samples, and allowed to set five minutes. The mass is then filtered, washed two or three times with hot water, the mass together with the paper is again transfered to the platinum dish, heated gently at first until all the water is driven off, then a strong heat from a Bunsen burner is applied until the whole mass becomes white. Allow to cool and then add the filtrate containng the salt and other soluble bodies, evaporate again to dryness, burn at a low red heat, cool and weigh.

$$\frac{(\text{Weight of Dish} + \text{Ash}) - \text{Wt. Dish}}{\text{Weight of Sample}} = \% \text{ Ash.}$$

RESULTS.

	++	++			++
Whippoorwill	Bread	Salt Pork	Feces	Butter	Milk
Peas					
<u>585</u>	<u>586</u>	<u>605</u>	<u>607</u>	<u>621</u>	<u>603</u>
Sample 2 Gm.	2 Gm.	2 Gm.	2 Gm.	4.0146	Pt. Dish
Wt. Cru.				Cru. +	= 14.3606
= 14.3618	14.3620	14.3560	14.3596	Ash = 16.1482	Pt. D. + Milk
Wt. Cr. + Sub.	16.3620	16.3560	16.3596	Ash = 16.3850	= 19.3858
= 16.3618				" + C .2368	M = 5.0252
Wt. Sample 2.	2.	2.	2.	" + Cr 16.3240	After hetng
Wt. Ash + Dsh				.1758	= 15.0563
= 14.4402	13.9440	14.4230	14.6068	% Ash 4.38	D = 14.3606
Wt. Dsh					Cas. + Ash
= 14.3618	13.3620	14.3560	14.3596		= .6962
Wt. Ash					C + A 14.3986
= .0784	.0320	.6700	.2472		" + 14.3906
% Ash					.0380
= 3.92	1.60	3.35	12.36		% Ash .76

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(2).

Whippoorwill	Bread.	SaltPork.	Feces.	Butter....	Milk.
Peas.					
<u>585.</u>	<u>586.</u>	<u>605.</u>	<u>607.</u>	<u>621.</u>	<u>603</u>
Sample					
2.Gm.	2.	2.	2.	3.8680	5.0216
Wt. Cru.				Cru+Ash	Pt Dish
13.8694	13.8694	32.7164	13.8666	18.9790	13.8646
Wt. Cru+Sub				Ash+	" +Milk
<u>15.8694</u>	<u>15.8694</u>	<u>32.7164</u>	<u>15.8666</u>	19.2036	<u>18.8862</u>
Wt Sample				Ash+Cas	Milk 5.0216
2.	2.	2.	2.	.2240	After hetng
Wt. Ash+Dish				Cru+Ash	Dish 14.8646
13.9482	13.9014	32.7836	14.1134	19.1460	Cas+Dish
Wt. Dish				Cru.	.6942
<u>13.8694</u>	<u>13.8694</u>	<u>32.7164</u>	<u>13.8666</u>	<u>19.9790</u>	Cru.+Ash
Wt. Ash				Ash	13.9034
.0788	.0320	.0672	.2468	.1670	Cru.
% of Ash				% Ash	13.8646
3.94 ✓	1.60	3.36	12.34	3.8680	Wt Ash
					.3880
					% of Ash
					.77

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Remarks.

44 The legumes and bread are determined in the usual way, but in the case of Salt Pork a slight variance is made, owing to the fact that so much salt is contained in the meat, which renders the loss greater unless certain precautions are taken. The following is the method; After the sample has been weighed it is placed on a hot plate, and heated for several hours at a low heat until most

of the volatile constituents have passed away and the contents reduced to a solid mass. The flame is then applied directing first at the top and gradually converging until the bottom is reached, and in this way loss is prevented. Again when the NaCl solution is added after the ash has become white great care is taken in evaporating, and when nearly dry, it is placed in an air bottle, heated to 100°C until constant and weighed.

(2). Great trouble is experienced in burning the Feces white, and it is sometimes necessary to burn 8 and ten hours, then again grind the contents with an agate pessle. If, however, in the initial burning, when all the organic matter has been consumed, the Bunsen burner flame is applied for a minute no throuble will be had in getting the Feces white.

(a). The sample of butter is melted in a closed vessel at as low a temperature as possible and when melted the whole is to be shaken violently for some minutes until the mass is homogeneous, and sufficiently solidified to prevent the separation of the water and fat. A sample of 5 cc is taken out through a pipette and measured into a weighed watch glass, then placed in the oven for twenty four hours until all the water is expelled, the total solids remaining. The dry butter from the water determination is transferred from the watch glass to a weighed Gooch crucible and contents are heated. The transfer from the watch glass to the crucible is accomplished by the use of a wash bottle filled with absolute Ether. The sample is then washed until free from fat. The crucible and contents are heated at the temperature of boiling

water until the weight is constant and the fat is calculated from the above data. The crucible containing the residue from the fat determination is covered and heated, gently at first, gradually raising the temperature to just below redness. The cover may then be removed and the heat continued until the contents of the crucible are white. The loss in weight of the crucible and contents represents Casein, and the residue the Ash.

(b). Milk. From the bulk sample of milk after being thoroughly shaken, 5 cc are measured off by means of a pipette into a weighed platinum dish and weighed. The milk is then evaporated to dryness and total solids obtained. The dry milk is then taken and heated until all the carbon is removed and the ash determined in the usual manner.

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Determination of Nitrogen.

Kjeldahl Method.

Preparation of reagents.

(a).

Saturated Sodium Hydroxide Solution.

Ten pounds of commercial Sodium Hydroxide free from nitrates are dissolved in 10 litres of distilled water and this makes a saturated solution.

(b). Granulated Zinc.

In order to prevent the flasks from bumping in the distillation of nitrogen, about 5 grams of granulated zinc, No 30, is added to the contents of the flask and thus the

trouble is avoided.

(c). Indicator. A solution of cochineal is prepared by digesting and frequently shaking 6 grams of pulverized cochineal in 100 cc of strong alcohol and 400 cc distilled water for a day or two at ordinary temperatures. The filtered solution is employed as indicator.

(d). Phenolphthalein. Two grams of Phenolphthalein are dissolved in 200 cc of alcohol.

(e). Preparation of $\frac{N}{5}$ Solution of Sodium Carbonate. A quantity say 20 grams of Kahlbaums pure sodium carbonate is placed in a clean platinum dish and heated for half hour to make sure all the moisture is driven off. Place in a dessicator until cooled. From this 10.6 grams are weighed and dissolved in one litre of and the solution is now ready for titration.

(f). Preparation of Normal Sulphuric Acid. This is made by taking 49 grams of pure Sulphuric Acid Sp. Gr. 1.84 (or 26.64 cc of H_2SO_4) and diluting to one litre. This is now titrated against the $\frac{N}{5}$ Sodium Carbonate Solution.

Standardizing of Normal Sulphuric Acid against $\frac{N}{5}$ Sodium Carbonate.

10 cc of normal H_2SO_4 are measured into a small beaker a few drops of phenolphthalein are added and the solution heated. The Na_2CO_3 is now allowed to pass in from a burette until about 49.5cc have been used, then the solution is heated and boiled for about five minutes to allow the excess of CO_2 to pass off. If no pink color appears a drop or two more are added and the boiling repeated.

This operation is repeated until exactly 50 cc of Na_2CO_3 have been neutralized and after the last drop has been added and the solution boiling a faint pink tinge only remains. It generally happens that the solutions do not balance at first and more H_2SO_4 or water must be added as the case may be.

(a). Check on the strength of the H_2SO_4 by precipitation of H_2SO_4 with Barium Chloride. The above titration with Na_2CO_3 is also checked by the Barium Chloride method, which consists in measuring 10 cc of the H_2SO_4 solution into a small beaker add 10cc HCl , 75 cc water, heat to boiling pour in boiling Barium Chloride. Evaporate for half an hour, ~~free~~ wash filter, wash free from Chloride and weigh as BaSO_4 . 1 cc = .0049 gr. SO_3 .

(b). Check by weighing as $(\text{NH}_4)\text{NO}_3$. 10 cc of standard Sulphuric Acid are pipetted into clean platinum dish which has been weighed, and 25 cc of strong NH_4OH are added. The contents of the dish is then gently evaporated nearly to dryness at a low heat. Then transferred to an air bath and dried at a temperature of 105°C for two hours. Then weighed and dried again with contents. 1 cc = .0049 grams SO_3 .

(c). Preparation of $\frac{N}{5} \text{NH}_3$. ____ grams (____cc) of Commercially pure Ammonium Hydroxide Sp. Gr. .96 are added and the solution diluted to 10 litres. The solution is then standardized using normal Sulphuric Acid. 10 cc of normal Sulphuric Acid are measured into an Erlenmeyer flask about 200 cc water added and about 2 cc of cochinal solution for an indicator. The NH_4OH solution is then allowed to pass in from a burette until 50 cc NH_4OH give a pink coloration to the solution. If it does not then more NH_4OH should

be added, or if too much is used more water until the NH_4OH and H_2SO_4 exactly balance.

(d). Potassium Sulphate.

This should be pure powdered potassium sulphate and free from nitrates.

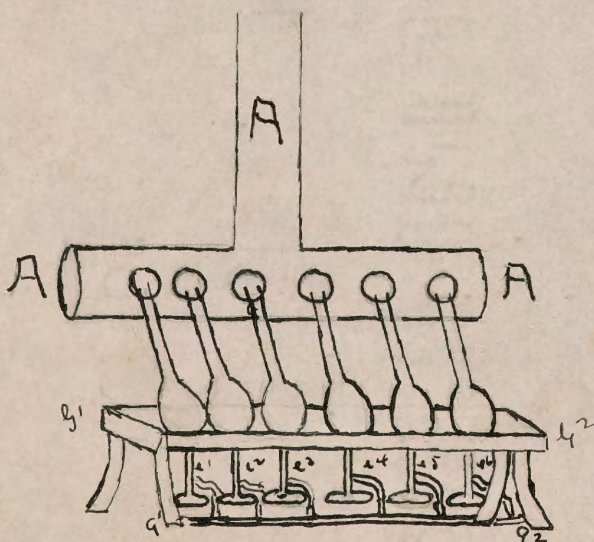
(e). Sulphuric Acid. This should be pure H_2SO_4 and free from Nitrates. It should have a Sp. Gr. 1.84.

Apparatus.

Kjeldahl Flasks were used for both digestion and distillation.

These are pea shaped round bottomed flasks having a capacity of about five hundred and fifty cc made of hard, moderately thick and well annealed glass. When used for distillation the flasks are fitted with rubber stoppers through which pass bulb tubes.

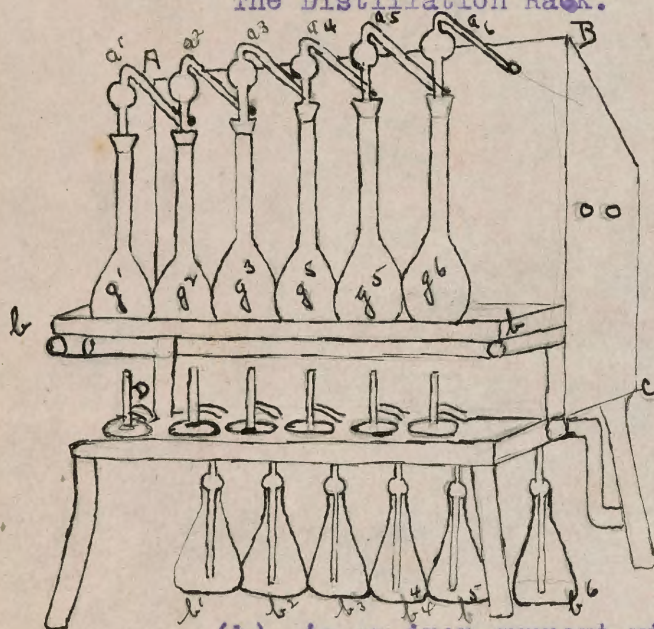
~~Rack of Distilling~~ ^{Digesting} Samples.



The digesting rack consists of a large hollow pipe A A A made of sheet lead placed upright on a pipe base of metal into which six round openings have been cut. Into these openings the necks of the flasks are fitted, the base resting on an iron rack, G' G2, especially made with six openings into which the round bottoms

of the flasks rest. Beneath each opening is a Boise burner, $e^1e^2e^3e^4e^5e^6$, connected to the main gas pipe $a' - a^2$ so that all the six may be lit at the same time or each separately. The SO_3 fumes pass of through the lead pipe a into the main flue and are carried off.

The Distillation Rack.

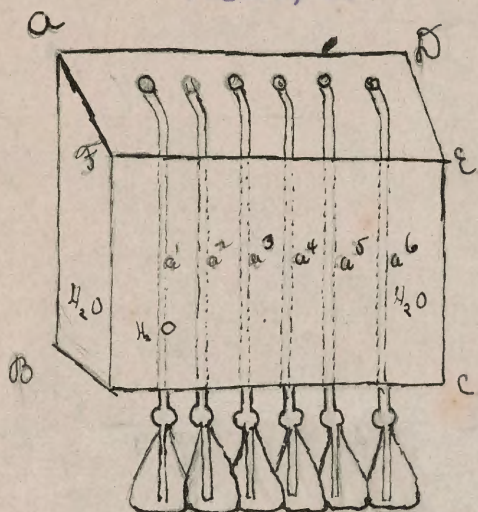


The distillation rack consists of a large tank A, B, C, D, filled with cold water and used as a condenser. Also six bulb tubes fitted with a rubber stopper and extending backwards through apertures, and connected to long metallic tubes $a^1 a^2 a^3 a^4 a^5 a^6$ projecting downwards into the flasks below.

(b), is an iron support with six openings so as to allow the distillations flasks, $g^1 g^2 g^3 g^4 g^5 g^6$ to rest. These are connected tightly to the bulbed tubes by means of rubber stoppers. c is another support used to hold the burners in place beneath the flasks.

Figure 2 represents the back view of the metallic tank A B C D. $a^1 a^2 a^3 a^4 a^5 a^6$ represent the metallic tubes, submerged in water, and in which the Ammonia is condensed and collected in the flasks $b^1 b^2 b^3 b^4 b^5 b^6$

Figure, 2.



below. A complete distillate is as follows: The flask containing the substance to be distilled is placed on the rack and heat applied. When all the NH_3 is driven over through the bulbed tubes into the cold metallic tubes, condensed and the distillate collected in the flasks below.

Estimation of per cent of Nitrogen in samples.

The Digesting. 2 grams of the sample are weighed in a platinum scoop and then transferred to a digesting flask. Ten grams of powdered potassium sulphate are added then 20 cc of concentrated H_2SO_4 . The flask is placed on the digesting rack and the heat applied, first gently, then raised until the acid boils. The boiling is continued until the contents of the flask have become a clear liquid or a pale straw color. The flask is now removed from the rack and allowed to cool, then it is ready for distillation.

The Distillation. After cooling about 200 cc of distilled water are added, the gram granulated zinc in order to keep the flask from bumping, then a few drops of phenolphthalein and finally Potassium Hydroxid, sufficient to make strongly alkaline, allowing it to run down the side so that it does not mix at once with the acid solution. The flask is now connected with the condenser and distilled until all the ammonia has been driven over into

the flask containing the 10 cc of the standard H_2SO_4 . The first 150 cc of the distillate will generally contain all the Ammonia and the distillation usually occupies from forty minutes to one and a half hours. To the distillate 2 cc of cochineal are added and then the solution is titrated with standard ammonia.

(50 cc — No. of ccs of Ammonia required) X 28

Weight of Sample

— % of N.

NOTROGEN DETERMINATION.

RESULTS.

(1).	Whippoorwill	Bread.	SaltPork.	Feces.	Butter.	Urine.
Peas.	585.	586.	605.	607.		591 a.
Sample	Sample	Sample	Sample	Sample		
2. Gm.	2.	2.	2.	2.	5.0234	5.1490
No ccs reqrd.	32.7	32.3	46.8	8.8	41.5	29.
% of N.=3.68	2.47	.45	5.76	.47	1.14	
.....						
(2).	Sample 2.	2.	2.	2.	5.0234	No Dup.
No. cc Rq.	23.8	32.4	46.8	8.9	41.5	
% of N.	3.67	2.46	.44	5.75	.47	

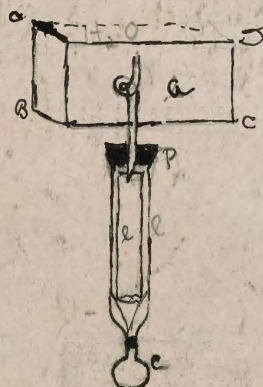
Remarks.

The length of time required in making the digestions of the different samples varies, especially between the legumes and feces, The former requiring two to three hours, while the latter six to eight. In making the determination of Urine and Milk, 5 cc of each were weighed into a platinum dish and then transferred to a digestion flask. The salt pork was weighed in a piece of filter paper and both substance and filter paper digested. In the distillation frothing was the only trouble encountered, and this was avoided by placing a piece of parafin in the flask before distillation.

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Determination of Water and Fat.

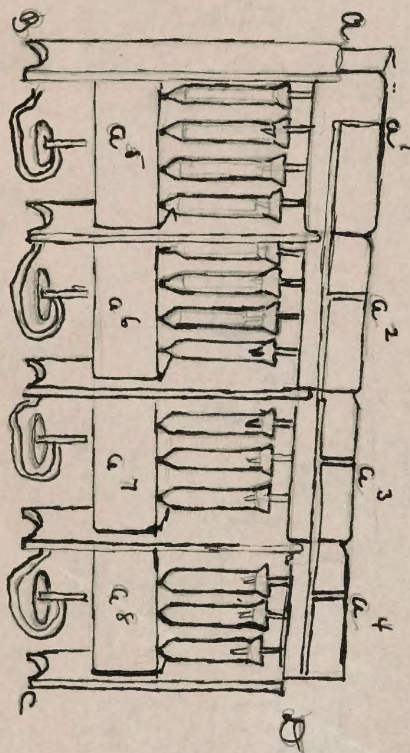
The fat extractor is so constructed that sixteen extractions may be made at the same time.



It consists of a rack A B C D , divided into four parts, each part holding four extractives, a a a a are metallic boxes filled with cold water and in the bottom of these are four openings through which pass small metallic

worms into the glass extractor beneath. The ether is forced up as a vapor by the worm in the tank a a a a a

and is condensed above, drops back again into the extractor, thus making a continuous distillation and condensing of the same ether.



A complete extractor consists (1) of a large glass extractor tube e, with a large opening at the top, while the other end tapers to a point and passing through a small cork. Inside of this large tube is a small cylindrical tube a open at one end, closed with linen and filter papers at the other, the closed end rests on small copper wires at the tapering end of the outer tube. The tank a contains cold water also the small worm extends through the bottom then through a cork up into the open end of the inner tube, c

is a small fat flask submerged in warm water, and fitted on to the cork through which passes the small end of the outer tube. Now when fat is to be extracted the substance is placed in the inner tube on the cloth and filter paper, then the tube placed in the outer tube, and ether poured on to the substance, and the whole attached to the cork p. The extracted fat goes into the flask c which, on account of being in warm water, vaporises the ether which passes through the outer tube into the worm r where it is condensed dropping back again on to the substance in

the inner tube and thus making a continuous ether dropping until all the fat has been extracted and caught in the flask below. The flask c is then taken out, the ether driven off weighed and the amount of fat calculated. The weight of c being previously known.

†† Estimation of Water and Fat.

Two grams of the substance are placed on a weighed watch glass, heated in the oven for about twelve hours until constant, cooled in dessicator and weighed ~~q_u~~ quickly so as to avoid absorption from the air. The sample after having been weighed is transferred to a fat extractor by means of a funnel washed well with ether and the extractor connected and allow to operate until all the fat is extracted and caught in the small fat flask. This flask having been previously weighed is now heated gently in oven to drive off ether weighed and per cent of fat calculated.

Results.						
# 1.						
Whip'will	Bread	Salt	Port	Feces.	Butter	Milk
Peas.						
585.	586.	605.	607	621		603.
Wt Glass						
7.3733	9.1038	10.3718	11.1680	14.3862		
Wt G. + Sub						
9.3733	11.1038	12.3718	13.1618	10.3716		
Wt Sub.						
2.	2.	2.	2.	4.0146		
Wt Dried						
9.2113	10.9780	12.2906	13.1012	13.7388		
Water free						
1.8380	1.8742	1.9188	1.9332	3.3672		
% " Free						
91.90	93.71	95.94	96.66	83.87		
# 2.						
Wt. Glass						
10.3732	8.8672	10.1522	10.3724	15.5944		
Wt. G + Sub						
12.3732	10.8672	12.1572	12.3724	11.1666	See Deter. Ash.	
Wt. Sub.						
2.	2.	2.	2.	4.4278		
Wt. Dried						
12.2120	10.7414	12.0688	12.3056	14.8791		
Water Free						
1.8388	1.8742	1.9166	1.9332	3.7125		
% W. F.						
91.94	93.71	95.83	96.66	83.85		
# 3.						
Wt Flask						
17.9900	14.6900	16.3182	17.4982			16.0682
" F. + Fat						
18.0170	14.7112	18.0976	18.0272			16.2962
" Fat						
.0270	.0212	1.7794	.5290			.2280
% of Fat						
1.35	1.06	88.97	26.45			4.54
# 4.						
Wt. Flask					See Ash	
16.0694	14.7967	17.0722	17.3500			12.6928
" F. + Fat					Determina	
16.0964	14.8133	18.8476	17.8766			12.9144
" Fat					tion	
.0270	.0216		1.7744			.2216
% of Fat						
1.35	1.08	88.77	26.35			4.41

Remarks.

xy Owing to the difficulty of extracting the feces with ether it is necessary to mix about twice its weight with clean, dry, washed sand, and then extract, thus preventing clogging of the extractor.

In the Milk extraction thick strips of filter papers are used. About five grams of milk are transferred to the paper by the use of a pipette, care being taken to hold the end of the paper in the hand so as to keep in dry. The paper is then rolled into a coil, tied with a silk thread, dried at the temperature of boiling water for an hour, then transferred to the fat extractive apparatus and the fat determined.

Heat of Combustion of Samples

xy The calories per gram in each sample of food were determined by Dr. Chas. E. Wait in the bomb calorimeter and will be published soon in a paper together with descriptions of methods and apparatus.