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THESIS

Composition of the Ash of the New Born Calf — E. E. Vannatta

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A STUDY
of the
COMPOSITION OF THE ASH

of the
NEW BORN CALF

by
Elsworth
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SUBMITTED IN PARTIAL FULFILLMENT OF THE
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A STUDY OF THE COMPOSITION OF THE ASH OF THE
NEW BORN CALF.

In making a chemical study of the composition of domestic animals of different ages and on different planes of nutrition, it is important to make a study of the composition of their ash and of the ash of similar animals at birth. This paper will deal with the composition of the ash of the new born calf.

A review of the work on ash analysis fails to show that any work has been done on the composition of the ash of the new born calf. A number of analyses have been made of the composition of the ash of different parts and organs of various animals and a few analyses have been made of the composition of the ash of entire animals at birth. In order that a comparison of the ash of the different parts of an animal, and of the composition of the ash of different animals may be made, some of the results secured by other chemists are here presented.

A. Jarische (1) reports the results of the analyses of the blood of a man, horse, cow and dog. His analyses will be of interest not only because they give the composition of the ash of the blood of a cow but also because they give the composition of the ash of the blood of other animals.

Composition of the Ash of Blood.

	Man	Horse	Cow	Dog.
P_2O_5	8.82	8.38	4.98	12.74
SO_3	7.11	6.31	6.17	4.13
Cl	30.74	28.63	35.12	32.47
K_2O	26.55	29.48	10.74	3.96
Na_2O	24.11	21.15	37.44	43.40
CaO	0.90	1.08	1.15	1.29
MgO	0.53	0.60	0.18	0.68
Fe_2O_3	0.16	9.52	9.24	8.64
CO_2		1.30	2.97	

Emil Abderhalden (2) also made a number of analyses of the ash of blood and compares his results with results previously secured by Bunge.

Composition of the Ash of Blood.

Weight in 1000 parts of blood	COW		HORSE
	Abderhalden	Bunge	Abderhalden
Na_2O	3.6350	3.631	2.091
K_2O	0.4070	0.411	2.738
Fe_2O_5	0.5440	0.577	0.828
CaO	0.0690	0.070	0.051
MgO	0.0356	0.036	0.064
Cl	3.0790	3.053	2.785
P_2O_5	0.4038	0.405	1.120
Total	8.1734	8.183	9.677

In order that these analyses may be comparable to the results secured by Jarische, the total per cent of ash in the blood and also the percentage composition of the ash of the blood has been computed.

Composition of the Ash of the Blood of the
Cow and Horse.

	Cow			Horse	
	Jarische	Abderhalden	Bunge	Jarische	Abderhalden
Na ₂ O	37.44	44.47	44.37	21.15	21.61
K ₂ O	10.74	4.98	5.02	29.48	28.29
Fe ₂ O ₃	9.24	6.64	7.05	9.52	8.56
CaO	1.15	0.83	0.85	1.08	0.53
MgO	0.18	0.44	0.44	0.60	0.66
Cl	35.12	37.67	37.31	28.63	28.78
P ₂ O ₅	4.98	4.94	4.95	8.38	11.57
SO ₃	6.17			6.31	
CO ₂	2.97				

Composition of the Ash of Bones.

J. W. Mallet (3) reports the analysis of Buffalo bones. Most of the bones from which the sample was taken had been exposed to the air a long time and were brittle. Results are given on bone dried at 105°C.

Composition of Dry Bone by J. W. Mallet.

Fat	0.788
Partially altered ossein	31.379
Ca ₃ P ₂ O ₈	54.058
Mg ₃ P ₂ O ₈	2.523
Ca F ₂	0.479
Ca Cl ₂	0.136
Ca CO ₃	8.250
CaO	0.782
NaCl	0.125
Fe ₂ O ₃	0.105
Insoluble siliceous residue	1.375
Na ₂ SO ₄	
KCl	trace.
Mn	

Dr. S. Gabriel (4) has done a large amount of work on analysis of the ash of bone. He obtained the following result on the analysis of the ash of the shank bone of a steer.

CaO	51.28
MgO	1.05
K ₂ O	0.18
Na ₂ O	1.09
Water of crystalization	2.33

P_2O_5	37.46
CO_2	5.06
Cl	1.04

E. Wildt (5) determined the composition of the ash of the bones of rabbits at different ages to determine if there was any change in composition due to age. The only change of importance was that the phosphate decreased while the carbonate increased.

Composition of the Ash of the Bones of
Rabbits by Wildt.

Age	CaO	MgO	P_2O_5	CO_2
Newly Born	52.17	1.38	42.05	3.65
Three days	52.16	1.36	42.13	3.84
Fourteen Days	52.10	1.26	42.19	3.99
One Month	51.91	1.22	42.20	4.00
Two Months	52.10	1.09	41.64	4.52
Three Months	52.49	1.01	41.03	4.69
Six Months	52.64	1.05	40.80	4.94
One Year	52.61	0.91	40.04	5.71
Two Years	52.76	0.93	39.78	5.81
Three to Four Years	52.84	0.83	39.80	5.66

Wildt did not report iron in the ash of bone.

Julius Katz (6) analyzed the ash of the flesh of a large number of animals and reported the results in detail. The following table gives the minimum and maximum in 1000 parts of flesh.

Composition of Ash of Flesh.

	Minimum	Maximum
K	2.4	4.6
Na	0.3	1.5
Fe	0.04	0.25
Ca	0.02	0.39
Mg.	0.18	0.37
Phosphorus from phosphates	1.22	2.04
Phosphorus from lecithin	0.13	0.48
Phosphorus from nuclein	0.09	0.32
Cl	0.32	0.80

H. Schulz (7) determined the sulphur in human and in various animal tissues. In the dried organs of a man, the percentage of sulphur varied from 0.57 in the brain to 1.03 in the jejunum. In the muscles of various animals, the percentage of sulphur varied from 0.86 to 1.33. Only traces of this sulphur are contained as sulphates.

H. Brubacher (8) made a large number of analyses of the bones, muscles, organs and of the entire bodies of three normal and of five rachitic children. The normal children (a) aged 28 weeks, (b) new born and (c) four years old.

The analyses evidently did not include all the elements in the ash.

Elements Reported in Ash of the Bones of
Children.

	a	b	c
CaO	48.09	48.99	53.82
MgO	1.44	1.28	1.55
P ₂ O ₅	40.21	40.68	42.58

Elements Reported in the Ash of Muscles.

	a	b	c
CaO	2.85	3.16	0.82
MgO	1.94	2.10	2.17
P ₂ O ₅	28.75	23.64	37.92
SiO ₂	1.36	0.35	1.80
Fe ₂ O ₃	1.16	1.39	0.75

Elements Reported in the Ash of the Entire
Body.

	a	b
CaO	34.82	36.37
MgO	1.56	1.42
P ₂ O ₅	36.33	36.77
SiO ₂	0.37	0.17
Fe ₂ O ₃	0.40	0.43

Abderhalden analyzed two new born guinea pigs and their mother's milk and found a close correlation between the composition of the ash of the newborn pigs and the mother's milk. Abderhalden first charred the sample, then extracted with water. He then reignited the residue and extracted with cold dilute nitric acid. The water and nitric acid solutions were combined and made up to a definite volume, and was designated as solution A. The portion not soluble in nitric acid was dissolved in hot hydrochloric acid, made up to volume and designated as solution B.

The table which follows gives the percentage composition of the ash in each solution for each animal.

Ash of Guinea Pigs and Ash of the Mother's
Milk.

	First Animal		Second Animal		Mother's
	Sol. A.	Sol. B.	Sol. A.	Sol. B.	Milk
K ₂ O	8.2216	7.9209	8.1565	8.0763	9.6926
Na ₂ O	6.9373	6.6002	6.9171	6.7044	8.9985
Cl	9.0968	9.9897	9.1119	9.6454	12.8421
Fe ₂ O ₃	0.2279	0.2456	0.2409	0.2366	0.1671
CaO	32.2136	32.9244	32.1758	31.9391	31.0705
MgO	3.0911	3.6323	3.3682	3.6677	3.0980
P ₂ O ₅	<u>42.2449</u>	<u>40.9307</u>	<u>42.0667</u>	<u>41.9056</u>	<u>37.0224</u>
Total	102.0332	102.2538	102.0371	102.1751	102.8912
deduct O-Cl	<u>2.0332</u>	<u>2.2538</u>	<u>2.0371</u>	<u>2.1751</u>	<u>2.8912</u>
	100.0000	100.0000	100.0000	100.0000	100.0000

Lawes and Gilbert (10) in their classical work give the complete composition of the ash of ten animals first of the entire carcass, second of the collective offal parts excluding the contents of the stomachs and intestines, third of the entire bodies of the ten animals excluding the contents of the stomachs and intestines.

Lawes and Gilbert

Percentage Composition of the Pure Ash of the Entire Bodies (excluding Contents of the Stomachs and Intestines) of Ten Animals.

Calculated to 100

	Fat Calf	Half Fat Ox	Fat Ox	Fat Lamb	Store Sheep	Half Fat Old Sheep	Fat Sheep					
							Analysis 1	Analysis 2	Mean	Very Fat Sheep	Store Pig	Fat Pig.
Fe ₂ O ₃	0.53	0.97	0.41	0.84	1.24	1.35	0.74	1.27	1.00	0.13	0.91	0.76
CaO	43.95	45.26	46.62	44.57	43.12	44.39	44.90	44.44	44.61	52.89	40.58	38.49
MgO	2.20	2.03	1.53	1.82	1.82	1.72	1.74	1.84	1.79	1.74	2.01	2.04
K ₂ O	5.40	4.41	4.46	5.74	5.64	5.27	5.57	5.50	5.53	0.37	7.39	8.57
Na ₂ O	3.82	3.08	3.04	3.58	3.90	3.35	3.53	3.59	3.56	1.71	4.16	4.36
P ₂ O ₅	40.37	40.22	39.80	38.96	38.96	39.15	39.08	38.56	38.72	40.61	40.12	40.14
SO ₃	1.08	0.86	0.79	1.18	1.78	1.06	0.99	1.03	1.01	0.32	2.33	2.15
CO ₂	1.34	1.97	2.13	1.53	1.09	1.83	1.35	1.67	1.67	2.02	0.60	1.20
Cl	1.55	1.24	1.47	1.86	2.31	1.61	1.61	1.60	1.61	0.22	2.22	2.78
SiO ₂	0.12	0.24	0.08	0.33	0.67	0.63	0.85	0.86	0.86	0.04	0.18	0.14
Total	100.36	100.28	100.33	100.41	100.53	100.36	100.36	100.36	100.36	100.05	100.50	100.63
Deduct O=Cl	0.36	0.28	0.33	0.41	0.53	0.36	0.36	0.36	0.36	0.05	0.50	0.63
Total	100.00	100.00	100.00	100.00	100.00	100.00		100.00	100.00	100.00	100.00	100.00

Experimental Part.

The calf used in this experiment was a normally developed pure bred Jersey calf which was strangled at birth. This calf weighed fifty-six pounds.

Preparation of Sample.

The entire calf including the contents of the stomachs and the intestines was cut into small pieces and run through a fine sausage mill many times, with thorough mixing between each grinding until the sample was homogenous. Small samples were weighed out in triplicate for various determinations, including total ash, total sulphur and total chlorine.

Total Ash.

Triplicate samples of about ten grams each were thoroughly dried and gently ignited in a tared porcelain crucible over a Fletcher burner until they were completely carbonized. They were then transferred to a muffle and heated to dull redness until the combustion of the carbon was complete. They were then cooled and weighed.

Total Weight of Ash in Calf.

Weight of Calf	Per cent of Ash	Total Weight of Ash	
Pounds		Pounds	Grams
5.60	4.07	2.279	1033.838

Drying the Sample Before Ashing.

A large sample was placed in an evaporating dish, saturated with redistilled alcohol and dried over a steam bath. The treatment with alcohol and subsequent drying was repeated until practically all of the water was removed.

Ashing.

Various methods for ashing meat have been suggested. Jarische (1) ashed samples of blood in a porcelain dish and claimed that the amount of silica which the ash gained from the dish was negligible.

Abderhalden (2) added barium hydroxide solution containing five grams of barium hydroxide to his sample, (He does not state the weight of sample taken) and evaporated to dryness on a water bath. The sample was charred with low heat, extracted with water, reignited and extracted with cold nitric acid. The insoluble residue was extracted with hot hydrochloric acid. The water and nitric acid solutions were combined and made to a definite volume. The hydrochloric acid solution was made to volume and a separate analysis was made of each solution.

Lawes and Gilbert ashed their samples in a large platinum dish heated in a muffle.

Preliminary attempts in this laboratory showed that it was impossible to ash meats in platinum dishes in a muffle without ruining the dishes. Therefore, the following method was employed. A basket three inches in depth and about

three inches in diameter with one-fourth inch mesh was made of platinum wire and suspended from a tripod by platinum wires. A large piece of glazed paper was placed under the tripod and under this glazed paper a larger piece of firm filter paper, to catch any scattered particles of the substance or ash. A casserole partly filled with the substance to be ashed was placed under the basket to catch the drippings which might fall from the basket.

The naked flame from a gas burner was directed against the sample. When the sample had ceased to drip the first casserole was replaced by a second which received the ash and fully carbonized material as it fell from the basket. Caution was observed to guard against loss from drafts.

When the first lot of the sample was fully carbonized, which usually did not take more than one hour, the second casserole was removed and the ash transferred to a suitable container. The scattered ash, if any and any portions of the sample which were on the paper, were carefully collected and added to the unashed part of the sample. The first casserole was again placed under the basket, the basket filled with the sample and the process of ashing carried on as before. Finally the portions of the sample in the first casserole were transferred to the basket and ashed. This operation carbonized all of the organic matter without removing all of the carbon.

The charred ash was next transferred to large platinum dishes and digested with water on a water bath for several

hours. The soluble portion was decanted through a filter into a large beaker, more water added to the insoluble ash and the ash again digested and decanted as before. This operation was repeated several times after which the ash was transferred to the filter and thoroughly washed with hot water.

The insoluble residue was transferred to the platinum dishes, carefully dried and then ignited to complete the combustion of the carbon. It was again digested and thoroughly washed as before. The filtrates were combined, concentrated, made up to five hundred cubic centimeters, and designated as the soluble ash. The water insoluble residue left after the second extraction was dried, reignited, weighed and designated as the insoluble ash.

Division of the Ash.

Weight of Soluble Ash.

Ten cubic centimeter samples of the soluble ash were drawn in duplicate and transferred to tared platinum dishes. The solutions were evaporated, heated below redness and weighed. The heatings and weighings were repeated until the weights remained constant.

Weight of soluble ash in large sample	6.6100 grams
Weight of insoluble ash in large sample	<u>66.8175 grams</u>
Total weight of ash in large sample	73.4275 grams

Per cent of ash soluble	9.002
Per cent of ash insoluble	90.998
Weight of soluble ash in entire calf	93.066 grams
Weight of insoluble ash in entire calf	<u>940.772 grams</u>
Total weight of ash in entire calf	1033.838 grams

Qualitative Examination of the Soluble and the Insoluble Ash.

Previous to making a quantitative determination, a qualitative examination was made both of the soluble and of the insoluble ash to determine the quantitative methods which should be employed.

Quantitative Analysis of the Soluble Ash.

Chlorine.

Samples in duplicate of ten cubic centimeters were drawn with a pipette and transferred to two hundred and fifty cubic centimeter beakers and diluted to one hundred cubic centimeters. The solution was made slightly acid by adding dilute nitric acid, and a slight excess of silver nitrate. The beakers were protected from the light and boiled until the precipitate had coagulated. The beakers were then covered with watch glasses and set in a cool dark place for a few hours..

The contents of the beakers containing the silver chloride were filtered on tared Gooch crucibles and washed with water containing a small amount of nitric acid. The Gooch crucibles were then heated in the oven one hour at 140° Centigrade, cooled and weighed..

Weight of Chlorine in Soluble Ash.

Aliquot	Weight of AgCl	Weight of Cl	Weight of Cl in Soluble Ash.
1/50	0.1157	0.02862	1.4310 grams
1/50	0.1157	0.02862	1.4310 grams
Average 1.4310 grams			

Samples in duplicate of five cubic centimeters were drawn with a pipette and transferred to two hundred fifty cubic centimeter beakers. Each sample was treated as follows. The sample was acidified by adding nitric acid in slight excess. A solution containing fifteen grams of ammonium nitrate was added and the sample raised to a temperature of 65°C. Sixty cubic centimeters of a solution of acid ammonium molybdate was added and the solution was digested at 65°C. for one hour. The beaker containing the ammonium phospho-molybdate precipitate was covered and allowed to stand over night. The sample was filtered and washed with a ten per cent solution ammonium nitrate. After all the chlorides were washed out the beakers which had contained the precipitate was placed under the filter and the precipitate was washed into the beaker by repeated washings with a hot solution of ammonia and water. To the solution hydrochloric acid was added in slight excess, then ammonium hydroxide in slight excess and the solution allowed to cool. Ten cubic centimeters of magnesia mixture was added drop by drop with vigorous stirring.

In fifteen minutes ten cubic centimeters of ammonium hydroxide (sp. gr. 0.90) were added and the sample allowed to stand over night. The solution was filtered through an ashless filter, the precipitate washed with a two and five-tenths per cent solution of ammonia. The filter with the precipitate was dried, transferred to a tared porcelain crucible, ignited and weighed as magnesium pyrophosphate.

Weight of Phosphorus in Soluble Ash.

Aliquot	Weight of $\text{Mg}_2\text{P}_2\text{O}_5$	Average	P_2O_5	P_2O_5 in Soluble Ash.
1/100	0.0103	0.0145	0.00665	0.665
1/100	0.0106			

Silica.

Silica, sulphuric anhydride, potash and soda were determined in the same sample but it would have been better to have taken separate samples for the silica, as a sample sufficiently large for a silica determination was larger than necessary for a potash or sodium determination.

Fifty cubic centimeter samples, in duplicate, of the soluble ash solution were drawn and transferred to platinum dishes, evaporated over a water bath, heated for an hour in the oven at $120^\circ\text{C}.$, cooled, moistened with strong hydrochloric acid, 20 c.c. of hot water added, again evaporated, heated, cooled moistened with hydrochloric acid, diluted with water, boiled and filtered through an ashless filter and thoroughly washed. The filtrate was dehydrated a second time. The precipitates were combined, dried, ignited in a tared porcelain crucible and weighed.

Total Silica in Soluble Ash.

Aliquot	SiO_2	Average	SiO_2 in Soluble Ash.
1/10	0.0050	0.0055	0.055
	0.0060	0.0055	

Sulphuric Anhydride.

The filtrates from the silica determination were combined, heated to boiling and an excess of a ten per cent solution of barium chloride was added drop by drop. The solution was kept at the boiling point for one hour, set aside over night. The precipitate was washed with hot water by decantation and on a double ashless filter. The precipitate was then dried, transferred to a tared porcelain crucible, ignited to whiteness and weighed as barium sulphate.

Total Sulphuric Anhydride in Soluble Ash.

Aliquot	Weight BaSO ₄ .	Weight SO ₃ .	Average weight in Soluble Ash.
1/10	0.2347	0.0805	0.813 grams
1/10	0.2395	0.0821	

Sodium.

The filtrate from the sulphuric anhydride determination was transferred to a platinum dish, evaporated over a water bath, heated below redness to remove the ammonium salts and dissolved in hot water. Barium hydroxide was added in excess, the solution was boiled, filtered and washed with hot water. The filtrate was heated to boiling, ammonium hydroxide and ammonium carbonate were added in excess and the solution allowed to stand a short time on the water bath.

The solution was then filtered and washed with hot water. The filtrate was evaporated to dryness and heated below redness. The residue was dissolved in a little hot water. A few drops of ammonium hydroxide, two drops of ammonium carbonate and a few drops of ammonium oxalate were added. The solution was warmed a few minutes, set aside a few hours, filtered and washed. The filtrate was again evaporated and heated below redness.

The residue was dissolved in a small amount of water and filtered into a tared platinum dish. A few drops of hydrochloric acid were added and the solution was evaporated to dryness. The residue was heated below redness, cooled and weighed as potassium and sodium chlorides.

Aliquot	Weight of Empty Crucible.	Weight of Crucible and Chlorides.	Weight of Total Chlorides.
	63.9169	64.5577	0.6408
	70.5400	71.1810	0.6410
		Average	0.6409

Potash.

The residue consisting of the total chlorides was dissolved in fifty cubic centimeters of hot water. A few drops of hydrochloric acid and platinum chloride solution in excess were added and the solution was evaporated to a thick paste on the water bath. The solution was then treated for at least one hour with eighty per cent alcohol. The precipitate was washed both by decantation and on a tared platinum Gooch crucible with eighty per cent alcohol until the filtrate

was colorless. The precipitate was next washed six times with ammonium chloride solution saturated with potassium chloroplatinate after⁺ it was thoroughly washed with alcohol. It was then dried in the oven at one hundred degrees centigrade and weighed as potassium chloroplatinate.

Aliquot	Weight Total Chlorides	Weight K_2PtCl_6	Weight K_2O	Weight KCl	Weight Na_2O
1/10	0.6408	1.3332	0.2588	0.4085	0.12328
1/10	0.6410	1.3302	0.2582	0.4094	0.12291
				Average	0.12309

Carbonates.

The addition of acid to a solution of the soluble ash caused no effervescence. When the solution was boiled and the gas conducted through lime water, the lime water became turbid. These tests proved the presence of a small amount of carbon dioxide in the soluble ash. The amount of normal hydrochloric acid required to titrate all the soluble ash in the cold to end reaction with phenolphthalein as determined by titrating an aliquot was 8.15 cubic centimeters. The amount of normal hydrochloric acid required to titrate all the soluble ash by the Farnsteiner method (17) as determined by titrating an aliquot was 10.41 cubic centimeters.

If the difference between these two titrations is regarded as representing the hydrochloric acid required to neutralize one-half of the carbonate, then it would require 4.52 cubic centimeters of normal hydrochloric acid to neutral-

ize all of the carbonate. The weight of carbon dioxide by this method was 0.0994 grams.

The weight of carbon dioxide as determined by absorption in soda lime in tared U-tubes was:

Aliquot	Weight U-tubes	Weight U-tube & CO ₂	Weight CO ₂
1/50	65.3093	65.3123	0.0030
1/50	65.3123	65.3149	0.0026
Average			0.0028

Percentage Composition of the Soluble Ash of the Entire Body (including the Contents of the Stomachs and Intestines) of the New Born Calf.

	Aliquot	Weight in the Aliquot grams	Weight in Total Solution grams	Percentage Composition of Soluble Ash.
K ₂ O	1/10	0.25850	2.5850	39.107
Na ₂ O	1/10	0.12309	1.2309	18.622
P ₂ O ₅	1/100	0.00665	0.6665	10.083
SO ₃	1/10	0.08130	0.8130	12.300
Cl	1/50	0.02862	1.4310	21.649
SiO ₂	1/10	0.00550	0.0550	0.832
CO ₂	1/50	0.00280	0.1400	2.118
			6.9214	104.711
Deduct O=Cl			0.3228	4.883
Total accounted for			6.5986	99.828
Undetermined				0.172

Composition of the Soluble Ash Referred to the Total Soluble Ash (93.066 grams) and to the Total Ash of the Calf (1033.838 grams).

	Composition of Soluble Ash Per Cent	Ratio of Soluble Ash to Total Ash	Constituents Referred to Total Ash Per Cent	Constituents Referred to Total Ash Grams
K_2O	39.107	9.002	3.520	36.391
Na_2O	18.622	"	1.676	17.327
P_2O_5	10.083	"	0.908	9.387
SO_3	12.300	"	1.107	11.445
Cl	21.649	"	1.949	20.150
SiO_2	0.832	"	0.075	0.776
CO_2	2.118	"	0.191	1.975
Total	104.711		9.426	97.451
Deduct = 0.01				
0=Cl	4.883		0.440	4.549
	99.828		8.986	92.902

The most difficult determination in the soluble ash was that of total solids. It required long heating at a temperature just below dull redness to expel all of the moisture. When the temperature was allowed to get too high part of the chlorides were volatilized and lost. To determine when all the moisture was expelled it was necessary to repeat the heating and weighing until constant weights were secured.

It was necessary to observe the same precautions in the determination of total chlorides. However, in the

determination of total chlorides it was not so difficult to secure constant weights.

Quantitative Analysis of the Insoluble Ash.

Silica.

A five gram sample of the insoluble ash was weighed into a six hundred cubic centimeter Erlenmeyer flask and boiled two hours in dilute hydrochloric acid. The acid soluble portion was filtered into a beaker. The precipitate with the filter paper was dried, ignited to whiteness, pulverized in a mortar, mixed with four times its weight of a molecular mixture of sodium and potassium carbonates and fused. The fused mixture (after cooling) was dissolved in hot water, acidified with hydrochloric acid and combined with the filtrate. The silica was removed and determined as described under the analysis of silica in the soluble ash.

Weight Sample	Weight Silica	Per Cent Silica
5.00	0.0318	0.63
5.00	0.0308	0.61
		Average 0.62

The filtrates from the silica determination were combined, made to a volume of two hundred and fifty cubic centimeters and designated as the solution of the insoluble ash. Phosphorus, iron, calcium and magnesium were determined in this solution.

Phosphorus.

Five cubic centimeter samples in duplicate (representing one-tenth of a gram of the insoluble ash) were transferred to two hundred fifty cubic centimeter beakers. Ammonium hydroxide was added in excess, the solution acidified with nitric acid and the analysis conducted as described under the determination of phosphorus in the soluble ash.

Weight Sample	Weight $Mg_2P_2O_7$	Weight P_2O_5	Per Cent P_2O_5
0.10 grams	0.06668	0.04248	42.48
0.10	0.04209	0.04209	42.09
		Average	42.29

Iron.

Preliminary tests proved the Zimmerman-Reinhardt method as described by Mixer and Dubois (11) to be the most satisfactory. It was found both convenient to manipulate and to give accurate results. Twenty-five cubic centimeter samples, in duplicate, of the solution of the insoluble ash were transferred to beakers and heated to boiling. A very dilute solution of stannous chloride (one to twenty-five) was added to the contents of each beaker until the color disappeared. Two or three drops were sufficient. The sides of the beaker were rinsed and the solution was diluted to about fifty cubic centimeters. Ten cubic centimeters of a saturated solution of mercuric chloride were then added. The solution was transferred to an Erlenmeyer flask which contained five hundred cubic centimeters of cold water and ten cubic centimeters of a preventative* solution and titrated with a potassium

permanganate solution; the end point was reached when the pink color disappeared in about fifteen seconds.

* The preventative solution was made by dissolving one hundred and sixty grams of manganous sulphate in seventeen hundred and fifty cubic centimeters of water, then adding three hundred and thirty cubic centimeters of phosphoric acid (sp. gr. 1.7) (syrupy) and three hundred and twenty cubic centimeters of sulphuric acid, (sp. gr. 1.82.

Weight of Sample	C.C. KMnO_4	Fe_2O_3 Factor	Weight Fe_2O_3	Per Cent Fe_2O_3
0.50 grams	3.20	0.001690	0.005408	1.08
0.50 "	3.30	0.001690	0.005577	1.12
0.10 "	0.92	0.0011221	0.00103	1.03
0.10 "	0.90	0.0011221	0.00101	1.01
0.10 "	0.98	0.0011221	0.00110	1.10
0.10 "	1.00	0.0011221	0.00112	<u>1.12</u>
Average				1.08

Calcium.

Twenty five cubic centimeter samples were drawn in duplicate and transferred to beakers. Each sample was analyzed as follows. The solution was neutralized with ammonium hydroxide, two grams of ammonium acetate were added and the solution was made strongly acid with acetic acid. The solution

was vigorously stirred and allowed to stand one hour when it was filtered and washed by decantation and on the filter with dilute acetic acid. The filtrate was removed and replaced by the beaker containing the original precipitate. The precipitate was washed into the beaker with hot dilute hydrochloric acid. A little dilute ammonium hydroxide was then poured on the filter. The dissolved precipitate was treated with ammonium hydroxide, and acetic acid and again filtered and washed. The precipitate was rejected.

The filtrates were combined and ammonium oxalate was added in excess to precipitate the calcium. The solution was boiled a few minutes and set aside a few hours. The precipitate was washed by decantation and on the filter.

The filtrate was replaced by the beaker which contained the original precipitate and the precipitate dissolved by pouring hot dilute hydrochloric acid through the filter followed by ammonium hydroxide solution. The solution containing the calcium oxalate was again treated with ammonium hydroxide, acetic acid and a few drops of ammonium oxalate, digested and filtered as before. The combined filtrates were set aside for the determination of magnesia.

A two hundred and fifty cubic centimeter measuring flask was placed under the filter, a hole punched through the filter and the precipitate washed into the flask with dilute sulphuric acid and hot water. The contents of the flask were allowed to cool and water was added to make up to volume.

An aliquot of the solution was drawn, diluted to

two hundred cubic centimeters and five cubic centimeters of sulphuric acid were added. The solution was heated to sixty degrees centigrade and titrated with a standardized solution of potassium permanganate.

Weight of Sample	KMnO ₄	Factor	Weight CaO	Per Cent CaO
0.10	11.95	0.003942	0.04711	47.11
0.10	11.95	"	0.04711	47.11
0.10	12.00	"	0.04730	47.30
0.10	12.05	"	0.04750	47.50
0.10	12.10	"	0.04770	47.70
0.10	12.10	"	0.04770	47.70
Average				47.38

Magnesium.

The filtrates from the calcium determination were combined and evaporated to about two hundred cubic centimeters. Five cubic centimeters of sodium phosphate were added and the solution was neutralized with ammonium hydroxide. The solution was stirred vigorously until a precipitate had formed, then set aside for fifteen minutes. Twenty cubic centimeters of ammonium hydroxide (sp. gr. 0.90) were added, the beaker was covered and set aside over night. The solution was filtered through an ashless filter and washed with a two and one-half per cent solution of ammonia. The filter and precipitate were dried, transferred to a tared porcelain crucible, ignited in the muffle to whiteness, and weighed as magnesium pyrophosphate.

"28"

Weight of Sample.	Weight of Mg P O	Weight of MgO	Per Cent of MgO.
1/2 gram	0.0316	0.0114	2.28
1/2 gram	0.0314	0.0114	2.28
Average			2.28

Sodium and Potassium.

A summation of the constituents found showed a shortage of 6.351 per cent in the water insoluble ash. The correctness of these determinations was verified. This proved that one or more of the constituents of the insoluble ash had not been determined. A qualitative examination was then made which showed that both sodium and potassium were present in the insoluble ash, although the insoluble ash had been washed until the filtrate was free from chlorides.

Sodium:

Sodium and potassium were then determined in the water insoluble ash by the J. Lawrence Smith method. A one-half gram sample of the ash was mixed in a mortar with one-half of a gram of ammonia chloride. Four grams of calcium carbonate were weighed out. The greater part of the carbonate was mixed with the sample in the mortar; the remainder of the carbonate was used to rinse the beaker. The mixture was transferred to a J. Lawrence Smith platinum crucible, the mortar rinsed with the carbonate and this added to the crucible. The crucible was heated gently until the fumes of ammonia were driven off. It was next heated at a high temperature for one hour, then cooled. Care was taken to heat

only the lower half of the crucible.

The contents of the crucible were transferred to a covered porcelain casserole and boiled thirty minutes with seventy-five cubic centimeters of water. The solution was decanted on a filter, more water added to the residue and the boiling repeated. The contents of the casserole were washed by decantation and on the filters. The filtrates were transferred to a casserole. Ammonium carbonate was added in excess and the solution evaporated to thirty cubic centimeters. Ammonium hydroxide and ammonium carbonate were added in excess and the solution was digested and filtered.

The filtrate was evaporated and heated below redness to drive off the ammonium salts. The residue was taken up with a small amount of water. A few drops of ammonium hydroxide, ammonium carbonate and ammonium oxalate were added and the solution was digested and filtered. The filtrate was evaporated and heated below redness. The residue was dissolved in a little hot water and filtered into a tared platinum dish. A few drops of hydrochloric acid were added and the solution was evaporated, heated below redness and weighed as potassium and sodium chlorides. Heating and weighing were repeated until the weights were constant. Blanks were run and corrections made for impurities in the reagents.

"30"

Weight Sample	Weight Total Chloride	Weight Blank	Weight KCl	Weight Na ₂ O	Per Cent Na ₂ O
0.50 gr.	0.0674	0.0105	0.0098	0.05000	5.000
0.50 gr.	0.0675	0.0105	0.00985	0.05002	5.002
Average					5.001

Potassium.

Potassium was determined by the method given in the analysis of the soluble ash.

Weight Sample	Weight PK ₂ PtCl ₄	Weight K ₂ O	Per Cent K ₂ O
0.50	0.0321	0.00625	1.25
0.50	0.0319	0.00620	1.24
Average			1.25

Composition of the Insoluble Ash Referred to the Weight of the Insoluble Ash (940.772 grams) and to the Weight of the Total Ash (1033.838 grams).

	Insoluble Ash Per Cent	Ratio of Insoluble Ash to Total Ash.	Constituents Referred to Total Ash Per Cent	Constituents Referred to Total Ash Grams.
K ₂ O	1.242	90.988	1.133	11.713
Na ₂ O	5.001	"	4.550	47.040
CaO	47.383	"	43.113	445.719
MgO	2.281	"	2.074	21.442
Fe ₂ O ₃	1.080	"	0.983	10.163
P ₂ O ₅	42.285	"	38.474	397.759
SiO ₂	0.620	"	0.564	5.831
99.892			90.891	939.667

Percentage Composition of the Entire Ash
of the New Born Calf (Including the Contents of the Stomachs
and Intestines).

	Soluble Ash Per Cent	Insoluble Ash Per Cent	Total Ash Per Cent
K_2O	3.520	1.133	4.653
Na_2O	1.676	4.550	6.226
CaO	none	43.113	43.113
MgO	none	2.074	2.074
Fe_2O_3	none	0.983	0.983
P_2O_5	0.908	38.474	39.382
SO_3	1.107	none	1.107
Cl	1.949	none	1.949
SiO_2	0.075	0.564	0.639
CO_2	0.191	none	0.191
Total	9.426	90.891	100.317
Deduct $O=Cl$	0.440		0.440
	8.986	90.891	99.877

Per Cent of Mineral Constituents in the
Entire Body (Including the Contents of the Stomachs and
Intestines) of the New Born Calf as Recovered in the Ash.

	Total Ash Per Cent	Total Ash in Calf Body, Per Cent	Composition of Ash in Calf Per Cent.
K_2O	4.653	4.07	0.1894
Na_2O	6.226	"	0.2534
CaO	43.113	"	1.7547
MgO	2.074	"	0.0844
Fe_2O_3	0.983	"	0.0400
P_2O_5	39.382	"	1.6028
SO_3	1.107	"	0.0450
Cl	1.949	"	0.0793
SiO_2	0.639	"	0.0260
CO_2	0.191	"	0.0078
Total	100.317		4.0828
Deduct $O=Cl$	0.440		0.0179
Total Found	99.877		4.0649

While small quantities of each of the mineral constituents may be lost in the process of ashing, probably with the exception of sulphur and chlorine the amount so lost (when proper precautions are observed) is negligible and will not seriously affect the results secured. Wiley (13) states that unless special precautions are taken a portion of organic

sulphur and phosphorus may be lost during combustion.

Sherman (14) conducted a series of experiments to determine the accuracy of various methods of determining sulphur and phosphorus in organic compounds, also to determine the amount of sulphur and phosphorus which remained when the substance was reduced to ash in the usual manner. In the phosphorus determinations he compared the accuracy of the Compressed Oxygen, the Sodium Carbonate and the Sulphuric Acid and Potassium Nitrate methods. He found the three methods to yield practically the same results. He compared these results with those secured by ashing the sample in the usual manner. In four analyses he found no loss by the latter method. In two other analyses the loss was small.

Loss of Sulphur in Ashing an Organic Substance
in the Usual Manner.

Fraps (15) conducted a series of experiments to determine the amount of sulphur recovered from the substance when ashed in the usual manner compared with that recovered by the Calcium Acetate Method. He found the loss of sulphur when ashed in the usual manner from four per cent to all the sample. Later, (16) he compared the Nitric Acid with the Calcium Acetate Method and found that the former gave the higher results. In a still later series of experiments he compared the Nitric Acid with the Sodium Peroxide or Osborne method, getting higher results with the latter.

Sherman (14) in a series of experiments compared the Compressed Oxygen, the Osborne (12) and the Nitric Acid

methods. The first two methods gave practically identical while the latter gave much lower results.

In order to determine the per cent of sulphur contained in the calf and the loss of sulphur by ashing in the usual manner, it was deemed best to determine the amount of sulphur in the sample by the Osborne Method (12).

Determination of Total Sulphur.

Ten grams of sodium peroxide were placed in a nickle crucible and just enough water was added to complete all reaction. The crucible was next heated until all the water was expelled. This was indicated by a scum on the liquid. The crucible was removed from the flame and partly cooled. A dried pulverized sample representing about 10 grams (accurately weighed of the original sample of the calf was added and thoroughly mixed with the contents of the crucible with a platinum stirring rod. The crucible was placed over the flame and the heating slowly continued with stirring of the contents of the crucible until all frothing had ceased and the mass had subsided. The heat was gradually raised. When the greater part of the carbonaceous material was destroyed, small quantities of sodium peroxide were cautiously added. If a snapping or flashing was observed, the liquid mass was heated further before adding more sodium peroxide.

Small quantities of the peroxide were added until the oxidation was complete. The fused mass was cooled and dissolved in water, transferred to a beaker, strongly acidified

with hydrochloric acid and boiled to drive off the excess of chlorine. The solution was filtered hot and washed until the filtrate was free from chlorine. The filtrate and washings were exactly neutralized with ammonium hydroxide and four cubic centimeters of concentrated hydrochloric acid were added. If necessary the solution was then evaporated to four hundred cubic centimeters. Ten per cent barium chloride was added in excess to the boiling solution. The solution was kept boiling at least half an hour, then allowed to stand at least twelve hours in a warm place. The precipitate was then filtered and washed with hot water containing a little hydrochloric acid and then with hot water till free from chlorides. It was then dried, ignited and weighed.

Weight Sample	Weight BaSO ₄	Weight S	Per Cent S.
10.8850	0.1683	0.0231	0.212
11.5705	0.1780	0.0244	0.211
11.8660	0.1841	0.0252	0.213
Average			0.212

The amount of sulphur trioxide in the entire body as recovered in the ash was only 0.045 per cent. This sulphur trioxide reduced to terms of sulphur amounts to only 0.018 per cent, showing a loss of 91.08 per cent of sulphur as compared to the Osborne method.

Determination of Total Chlorine.

In order to determine the amount of chlorine originally present in the calf and the per cent lost in ashing, a determination of chlorine was made in the following manner.

Samples in triplicate weighing about ten grams each were weighed from the fresh prepared sample of the calf and dried in an oven. The sample was all transferred to an agate mortar and pulverized. Calcium oxide was added in the proportion of five to one and thoroughly mixed with the sample. A little calcium oxide was first put into a hard glass tube forty centimeters in length and fifteen millimeters in diameter sealed at one end. The sample was next transferred to the tube. Then more calcium oxide was added to the contents of the tube. The tube was clamped in a sloping position to a ring stand and ignited first at a low, afterwards at a high heat. The heating was continued until the sample was completely carbonized, which took about one hour. The heat was first applied to the bottom of the tube, gradually heating higher up on the tube.

When the tube had cooled, its contents were transferred to a beaker, dissolved in water and acidified with nitric acid. The contents of the beaker were filtered and washed. Chlorine was determined in the filtrate as described under determination of chlorine in the soluble ash. One of the samples was lost during the determination. The results from the other samples were as follows:

Weight Sample	Weight AgCl.	Weight Cl.	Per Cent Cl.
10.1445	0.0866	0.0214	0.211
9.8310	0.0831	0.0208	0.209
Average			0.210

The per cent of chlorine in the entire body as recovered in the ash was only 0.0793, showing a loss of 62.24 per cent of chlorine during the process of ashing.

Summary and Discussion.

It will be of interest to compare the composition of the ash of the new born calf to that of older animals as determined by Lawes and Gilbert.

Percentage Composition of the Ash of the Entire Body (including the Contents of the Stomachs and Intestines) of the New Born Calf as Determined.

Percentage Composition of the Ash of the Entire Body (excluding the Contents of the Stomachs and Intestines) by Lawes and Gilbert.

Calculated to one hundred per cent.

	New Born Calf	Fat Calf	Half Fat Ox	Fat Ox.
Fe ₂ O ₃	0.983	0.53	0.97	0.41
CaO	43.113	43.95	45.26	46.62
MgO	2.074	2.20	2.03	1.53
K ₂ O	4.653	5.40	4.41	4.46
Na ₂ O	6.226	3.82	3.08	3.04
P ₂ O ₅	39.382	40.37	40.22	39.80
SO ₃	1.107	1.08	0.86	0.79
CO ₂	0.191	1.34	1.97	2.13
Cl	1.949	1.55	1.24	1.47
SiO ₂	0.639	0.12	0.24	0.08
Total	100.317	100.36	100.28	100.33
Deduct O-Cl	0.440	0.36	0.28	0.33
Total	99.877	100.00	100.00	100.00

A comparison of the composition of the ash of the new born calf with that of older animals as determined by Lawes and Gilbert shows the ash of the new born calf to contain a higher per cent of iron, sodium, sulphate, chlorine and silica than the ash of the older animals,

The per cents of magnesium and of potassium do not differ more than in the older animals. The per cents of calcium, phosphorus and carbon dioxide are lower in the ash of the new born calf.

As shown by Jarische (1) and Abderhalden (2) the ash of blood contains a high per cent of sodium, iron, and chlorine. Constituents which are found to be higher in the ash of the new born calf than in the older animals. As shown by Mallet (3), Gabriel (4), and Wildt (5) the ash of bone is composed almost entirely of calcium phosphate. These constituents are found to form a lower per cent of the ash of the new born calf than of older animals. As shown by Katz (6), the ash of flesh consists very largely of potash and phosphorus.

The ash of the new born calf contains less potash than the ash of the six months old fat calf but more than the ash of the older animals. The per cent of phosphorus is lower in the ash of the new born calf than in the ash of older animals.

As has been shown, the amount of chlorine and sulphur recovered in the ash is not a true measure of the amount of these constituents which were present before the sample was reduced to ash. The amount of silica reported in the ash

(0.639 per cent)
of the new born calf is much higher than the amount found by Lawes and Gilbert. In their work they reported both the analysis of crude ash and of pure ash calculated to one hundred per cent. In their analysis of crude ash they report sand and silica as follows.

	Sand	Silica
Fat Calf	0.35	0.12
Half Fat Ox	0.98	0.24
Fat Ox	0.69	0.08

In the analysis of the insoluble ash as described there was a residue, insoluble in boiling hydrochloric acid which fused and added to the solution. If this insoluble residue had been reported as sand, the per cent of silica would probably have been comparable with that found by Lawes and Gilbert.

The amount of carbon dioxide found in the ash of the new born calf was much lower than that found by Lawes and Gilbert. Probably caused by lack of basic elements with which to combine. It is probable that the composition of the ash of flesh or bones of different animals of the same age and species would vary as widely as has the percentage of the constituents which may be considered as essentially those of flesh and bones.

The high per cent of iron and sodium indicates that the ash of the new born calf contained a larger amount of the mineral constituents of blood than did those of the older animals.

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