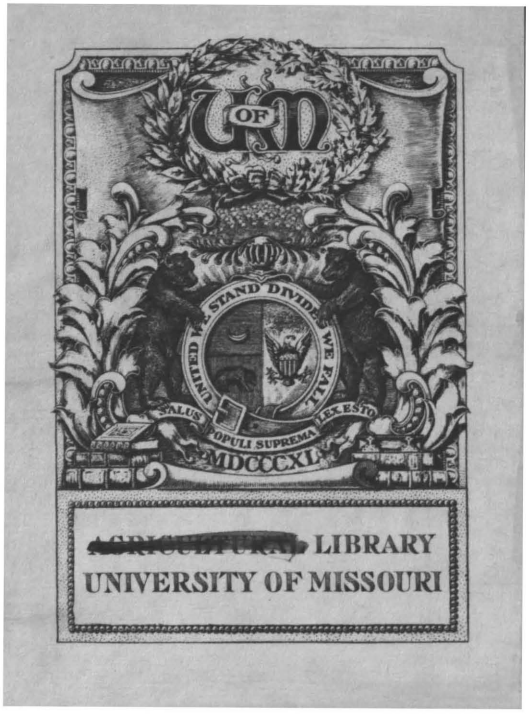

A STUDY OF THE CHEMICAL COMPOSITION

of a

FULL-TERM BOVINE FOETUS

by

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The Development of the Bovine Foetus.

Historical

Attention has been frequently directed to the prenatal development of various animals, as well as of man. This embryonic development of the bovine foetus is to be the subject of special investigation by the Agricultural College of the University of Missouri, and this analysis of a new born calf is intended as one of a series of contributions on that point. Most analyses of this nature have been confined to the mineral constituents, at least parallel work is not available here. In fact, very little similar work of any kind has been done on domestic animals. Still a comparison of work similar in any respect is interesting and may be of value, although the composition of a new born calf, or of a calf embryo, may be slightly different from that of any other specie.

Mr. E. E. Vanatta of this Station made a complete analysis of the mineral constituents of a new born calf last year; his results will be discussed later.

L. Hugounenq [1] reports the ash analysis of a human foetus which had attained full term.

Composition of the Ash of a Human Foetus.

Constituents	In 100 parts ash.
P_2O_5	35.28
CaO	40.48
MgO	1.51
Cl	4.26
SO_3	1.50
Fe_2O_3	.39
K_2O	6.20
Na_2O	8.12
CO_2	1.89
	99.63

The author points out that the calcium is in excess over the phosphoric acid, which would be in the acid phosphate state unless the other bases intervened. Furthermore, the soda predominates over the potash, as there are about two molecules of soda to one of potash. In the young of the dog, cat, rabbit, the potash ordinarily predominates.

In another article,[2] the same author gives the per cent of ash, also of iron in human embryos of different ages.

Age of Foetus	Sex	Weights of Foetus kg.	of Ash grams	Fe_2O_3 for Total Organism grams	for 100 parts of ash, gr.
4.5 months	F	.522	14.0024	.060	.432
5 "	F	.570	18.7154	.061	.327
5 "	F	.800	18.3572	.073	.400
5-5.5 "	F	1.115	28.0743	.106	.378
5.5 "	F	1.285	32.9786	.126	.383
6 "	F	1.165	30.7705	.119	.387
Full term	M	2.220	96.7556	.383	.396
Full term	M	3.300	106.1630	.421	.397

The author concludes in this paper that the fixation of the mineral elements is slow at first, as the salts fixed by the foetus in the last three months are about twice those fixed in the first six months. The fixation of the iron is about the same as that of the other elements so far as time is concerned.

Analyses similar to those of Hugounenq[1] are reported by Camerer.[3] Two new born children were analyzed, and the results given here are parts in one hundred of ash.

	Child I	Child II
K_2O	8.9	6.8
Na_2O	10.0	8.3
CaO	33.5	38.7
MgO	1.3	0.6
Fe_2O_3	1.0	0.7
P_2O_5	37.7	40.2
Cl	8.8	6.6
Sum	101.2	101.9
O= Cl	2.0	1.5
	99.2	100.4

The mineral composition is also given, referred to 100 grams body substance.

	Child I	Child II
K_2O	0.18	0.18
Na_2O	0.21	0.22
CaO	0.70	1.04
MgO	0.03	0.02
Fe_2O	0.02	0.02
P_2O_5	0.78	1.07
Cl	0.18	0.18
Sum	2.10	2.73
O=Cl	0.04	0.04
	2.06	2.69

An ash analysis of a new born infant is reported by Söldner [4]. In the same paper, he compares his results with those of other investigators.

100 parts ash contains

Constituents	Söldner	Hugounenq	Michel	de Lange	Giacosco
K_2O	7.06	6.20		6.54	2.70
Na_2O	7.67	8.12		8.80	10.23
CaO	38.08	40.48	41.39	38.89	41.92
MgO	1.43	1.51	1.20	1.37	1.10
Al_2O_3	0.11				
Fe_2O_3	0.83	0.39		1.69	1.89
Mn_3O_4	0.03				
P_2O_5	37.66	35.28	38.02	37.61	37.65
SO_3	2.02	1.50			
Cl	6.61	4.26	5.73	6.36	5.77
SiO_2	0.06				
Co_2	0.53	1.89			
Sum	102.09	99.63		101.26	101.26
O=Cl	1.49	0.96		1.43	1.30
	100.60	98.67		99.83	99.96

Perhaps the most interesting points here are the high per cents of iron reported by de Lange and Giacosco, also the low per cent of potassium given by the latter.

In a later paper Camerer[5] reports additional data on new born infants.

100 grams body substance contains

	Water	Dry Substance	Fat	Ash	Protein	Extractives
Average of four	71.7	28.3	12.8	2.6	11.5	1.4

The high per cent of fat is noteworthy and may account for the fact that he reports a lower per cent mineral matter than does Fehling.

Two of the specimens used were male, and two were female. He has arranged the figures in another table to show that sex does not influence the chemical composition.

The same author contributes more complete data in a later article[6]. Only his ash analysis is given here, however,

Relative figures.

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	Cl
100 grams body substance contain	0.19	0.23	1.01	0.03	0.018	1.02	0.18
100 grams ash contain	7.1	8.6	37.9	1.01	0.6	38.2	6.6

He concludes again that sex has no important influence on the chemical composition.

Abderhalden[7] had made investigations similar to those reproduced here and in one publication[7], he gives a partial analysis of new born guinea-pigs. As the results agree well, only one analysis is given here.

The new born animal contains in 100 grams body weight:

K_2O	0.2884	grams
Na_2O	0.2435	"
Cl	0.3193	"
Fe_2O_3	0.0080	"
CaO	1.1307	"
MgO	0.1085	"
P_2O_5	1.4828	"
Sum of ash content	3.5812	"
O=Cl	0.0720	"
	3.5092	"

Of the four ash analyses, one representative determination is included here.

100 parts ash contain	
K_2O	8.1565
Na_2O	6.9171
Cl	9.1119
Fe_2O_3	0.2409
CaO	32.1758
MgO	3.3682
P_2O_5	42.0667
Sum of ash content	102.0371
O=Cl	2.0371
	100.0000

In as much as this investigation is intended as one of a series which shall include embryos of different ages, some of Fehlings results[3] might well be given here. He collected a number of human embryos of different ages and made partial analyses.

No.	Length in Cm.	Sex	Age	Absolute weight in Grams		In per cent of Total Mass			
				Fresh	Dry	Water	Ash	Fat	Protein
1	2.5	M	6 weeks	0.98	0.24	97.54	.001	—	—
2	12.0	K	4 months	36.5	3.00	91.79	.98	0.57	4.87
3	13.5	K	4 "	56.5	5.1	90.97	1.01	0.45	5.24
4	18.5	K	1st half 5 months	95.5	8.9	90.70	1.4	0.48	5.9
5	18.5	K	5 "	104.7	8.2	93.20	1.04	0.51	5.6
6	19.0	M	2nd half 5 months	156.8	14.6	90.7	1.43	0.54	6.0
7	21.5	K	5 "	244.0	22.0	90.96	1.16	0.28	7.1
8	22.5	K	5 "	235.5	24.0	89.81	1.64	0.57	6.4
9	23.0	K	5 "	264.0	29.5	88.9	1.89	0.52	7.7
10	24.0	K	5 "	299.0	32.8	89.3	1.91	0.6	7.3
11	26.0	K	6 "	361.8	39.1	89.2	1.94	0.72	6.67
12	30.0	M	6 "	575.0	79.5	86.4	2.33	1.06	7.8
13	33.5	K	6 "	771.0	125.2	83.77	2.84	1.98	8.87
14	34.5	M	7 "	910.0	159.0	82.6	2.94	3.47	11.8
15	34.0	K	7 "	832.9	138.2	83.5	2.28	2.7	11.4
16	36.0	M	7 "	836.0	136.5	83.9	2.85	2.21	11.1
17	35.0	K	7 "	1117.0	170.71	84.8	2.54	2.36	9.1
18	38.0	K	8 "	928.0	159.5	82.9	2.82	2.44	10.4
19	53.5	K	Mature	3294.0	855.52	74.1	2.55	9.1	11.8
20	44.0	K	9 months	1760.6	456.1	74.7	3.3	8.7	12.6
21	45.0	K	9 "	1495.7	391.2	73.9	2.11	5.11	17.8

A consideration of this table shows a very high moisture content in the early stages, which gradually diminishes throughout the foetal period. There is evidently an accumulation of fat also in this period, gradual at first, more rapid

later. If the calculations are made on a dry basis, it is evident that the per cent of protein in the early stages must be greater than in the later stages. The same calculation will also show that the per cent of mineral constituents in the dry matter is about the same throughout the period.

A number of analyses of new born children have been reported by Brubacher[8] which are somewhat similar in nature. Of the three specimens analyzed, the first was prematurely born in the twenty-eighth week. The second was born dead in the thirty-sixth week, and the third died of diphtheria when four years old, though in a good state of nutrition.

	Bones.					
	In 100 parts fresh organ			In 100 parts dry fat free organ		
Constituents	Skeleton I	Skeleton II	Ribs III	Skeleton I	Skeleton II	Ribs III
Water	72.21	___	45.02			
Fat	0.59	___	2.55			
Ash	11.28	___	27.87	41.47	48.76	53.16
CaO	5.42	___	15.00	19.94	23.89	28.61
MgO	0.16	___	0.42	0.60	0.62	0.82
P_2O_5	4.53	___	11.87	16.67	19.84	22.63

Constituents	In 100 parts ash		
	Skeleton I	Skeleton II	Ribs III
Water			
Fat			
Ash			
CaO	48.09	48.99	53.82
MgO	1.44	1.28	1.55
P_2O_5	40.21	40.68	42.58

Portions of the second specimen are not analyzed. The entire skeleton of the third was not analyzed, so the

author assumes that the composition of the ribs would be similar to that of the entire skeleton.

This station, however, has made a number of similar analyses on the bones of beef animals. The data is as yet unpublished, but shows clearly that no single portion of the skeleton can be taken as typical of the entire skeleton. For that reason the value of the comparison Brubacher makes is open to question.

An analysis of the muscles is also given.

Constituents	In 100 parts fresh organ			In 100 parts fat free dry organ		
	I	II	III	I	II	III
Water	83.93	___	77.24			
Fat	2.25	___	1.81			
Ash	1.03	___	1.02	7.47	6.39	5.30
CaO	0.03	___	0.01	0.21	0.20	0.04
MgO	0.02	___	0.02	0.14	0.14	0.11
P_2O_5	0.26	___	0.39	1.89	1.51	2.01
SiO_2	0.01	___	0.02	0.01	0.02	0.10
Fe_2O_3	0.01	___	0.01	0.09	0.09	0.04

	In 100 parts ash		
	I	II	III
Water			
Fat			
Ash			
CaO	3.85	3.16	0.82
MgO	1.94	2.10	2.17
P_2O_5	28.75	23.64	37.92
SiO_2	1.33	0.35	1.80
Fe_2O_3	1.16	1.39	0.75

Entire Body.

Constituents	In 100 parts fresh organ I	In 100 parts fresh organ II	In 100 parts fat free dry organ I	In 100 parts fat free dry organ II	In 100 parts ash I	In 100 parts ash II
Water	80.75	75.28				
Fat	3.95	8.42				
Ash	3.00	3.12	19.60	19.11		
CaO	1.04	1.13	6.83	6.95	34.82	36.37
MgO	0.04	0.04	0.30	0.28	1.56	1.42
P_2O_5	1.09	1.15	7.12	7.03	36.33	36.77
SiO_2	0.01	0.01	0.07	0.03	0.37	0.17
Fe_2O_3	0.01	0.01	0.08	0.08	0.40	0.43

The analysis of the skeletons would indicate that the per cent of fat in bones is much higher after birth than before, even if the calculation is made on a dry basis; this is also true of the per cent of ash, calcium and phosphorus. The bone also contains a higher per cent of calcium and phosphorus after birth.

In the muscle analysis several points seem worthy of note. The per cent fat is higher in No. I than in No.III, judged by the analysis of the fat free dry tissue, the per cent of ash and of calcium decreases with age. Apparently no conclusion can be drawn concerning the phosphorus, though the amount of iron seems to diminish.

The analysis of the entire body indicates a higher per cent of fat in the more advanced foetus. The per cent of ash, calcium and phosphorus is about the same. There

seems to be a slightly higher per cent of calcium and phosphorus in the ash itself, though individual differences alone could easily account for that.

A paper by Margaret Wilson[9] contains an analysis of three new born pigs.

	Live Weight	Solids	Fat	Fat Per cent	Proteid	Proteid Per cent	CaO	CaO Per cent
Pig I	1044	205.1	13.70	6.68	127.3	62.6	19.28	9.40
Pig II	1142	226.4	17.65	7.79	136.1	60.1	—	—
Pig III	1016	207.9	16.21	7.79	127.1	61.1	19.52	9.39
Average				7.42		61.3		9.40

Some interesting results are reported by Krüger which may have a certain value, although similar analyses were not made in this investigation. He collected the liver and spleen from a large number of bovine embryos, and determined the content of a number of the elements. In the first paper[10] he gives the iron analysis. The average iron content of the liver cells for the entire intra-uterine period is about 0.2801 per cent of the dry substance, or about ten times that of the mature animal. The greatest iron percentage is at a length of 20 - 30 cm. This slowly sinks until a length of 40 - 50 cm. is attained, when the iron content is at a minimum. There is an increase again until the embryo is 70 - 80 cm. long, and then a rapid decrease until birth. After birth the iron content continues to fall.

Iron Content of Liver Cells.

.20 - 30 cm.(5)		30 - 40 cm.		40 - 50 cm.		50 - 60 cm.(6)		60 - 70 cm.(7)		70 - 80 cm (8)		80 - 90 cm.(9)	
No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %
4	0.3586	6	0.2143	19	0.1402	20	0.1814	16	0.2960	10	0.3092	6	0.1809

The figures in parenthesis above are to represent the approximate age - in months - of the foetus, as given by the same author in a subsequent paper[11].

Iron Content of Liver Cells.

Calves								Oxen		Cow	
One Week		Two Weeks		Three Weeks		Four Weeks		Three years		Pregnant	
No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %	No.	Iron %
10	0.1800	5	0.0863	3	0.0496	3	0.0322	5	0.0246	6	0.0276

Concerning the analysis of the spleen, the author notes that the females have five or six times as much iron in that organ as the males. Also, the amount of iron in the spleen is much lower in the foetal period than in maturity.

In a later publication[11] Krüger gives the calcium content at different ages.

Calcium Content of Liver Cells.

No.	20 - 30 cm.	No.	30 - 40 cm.	No.	40 - 50 cm..	No.	50 - 60	No.	60 - 70 cm.	No.	70 - 80 cm.	No.	80 - 100 cm.	No.	Mature animals
8	0.058	13	0.101	16	0.081	11	0.082	11	0.064	6	0.078	10	0.104	14	0.123	8	0.071

Krüger also analyzed the liver and spleen for sulphur and phosphorus[12]. The sulphur content is about the same at all periods of life. The phosphorus varies somewhat more for it is somewhat higher in the embryonic stages, and lower after birth, especially after maturity is reached.

The amount of glycogen in embryonic tissues has been investigated somewhat on this point. The results of Mendel and Leavenworth[13] are perhaps most suitable for citation here. Their researches were conducted with pig embryos. They found a small amount of glycogen in embryonic tissue. The distribution of glycogen in embryonic structures was about the same as in the adult. The liver began its glycogen storing function late in the prenatal period. They considered the metabolism of glycogen in the embryo comparable with that in the adult.

Experimental.

The calf used for analysis was a normally developed Jersey, weighing ~~seventy-two~~ 704 pounds. It was carried full time, but strangled at birth.

As soon as possible it was taken to the laboratory and prepared for analysis. The abdominal and thoracic cavities were opened as nearly as possible on the median ventral line, the internal organs removed and each weighed separately. The solid contents of the large intestines were removed and weighed separately as excreta. The soft contents of the intestines were left in. The dissection was then continued so as to divide the specimen as nearly as possible into two equal parts, right and left. The tongue, trachea, oesophagus, left kidney, and central nervous were removed as completely as possible; with those exceptions, the left side was discarded and the right side preserved for analysis. The skin was removed, then the flesh separated from the skeletal parts. It was impossible to collect the blood as is done when an animal is slaughtered, and besides that, a small amount of water was used to wipe up dried blood, and for similar purposes; so the exact weight could not be obtained. However, all liquids, blood, serum, and the water used, were wiped up in clean cloths previously weighed, reweighed, then the liquids wrung out of the cloths and preserved for analysis. The weights of the various portions are as follows:

Division of Animal.

	Half Total Weight	Total Weight
Blood, serum, and water		1675 grams
Liver		1581 "
Nervous system		338 "
Lungs and trachea		218 "
Market heart		255 "
Circulatory system		169 "
Stomachs		467 "
Intestines		1215 "
Excreta		301 "
Spleen		124 "
Thyroid		120 "
Omentum		168 "
Right kidney		86 "
Left kidney		107 "
Market tongue		170 "
Base of tongue		67 "
Skin	1948 grams	3896 "
Skeleton	3777 "	7554 "
Flesh	6431 "	12862 "
	Total	31373 "

The weight of the calf as obtained before dissection was ~~22.0~~ 70.4 pounds. The total weight as calculated back from the weight of the individual organs is 69.2 pounds. The discrepancy is probably due partly to evaporation, and partly to the difficulty of making an exactly equal division of the animal in dissection.

The following samples were then prepared for analysis:

11-12-1	Blood, serum
11-12-2	Liver
11-12-3	Nervous system
11-12-4	Internal organs
11-12-5	Hide
11-12-6	Skeleton
11-12-7	Flesh
11-12-8	Fat from left kidney
11-12-9	Excreta
11-12-10	Composite of entire animal, including excreta.

The internal organs comprise those portions of the animal given in the previous lists which do not appear here for separate analysis. The blood was ready for analysis after thorough mixing. The bones could not be finely ground while in the fresh condition, so they were broken up as finely as possible in a meat chopper. The other portions were each ground separately in a sausage mill until finely divided, then thoroughly mixed. Portions of each sample were taken and thoroughly mixed for a composite. Each of the ten samples was analyzed for moisture, fat, ash, phosphorus, and nitrogen. In addition, 3600 grams of the composite were weighed out for a complete mineral analysis. Samples of the composite were also weighed out for determining total sulphur and total chlorine. All samples for all

determinations were weighed out immediately, in the containers in which the determinations were to be made. All analyses were made in triplicate. Since the bones could not be finely ground while fresh, large samples were weighed out in evaporating dishes, and moisture and fat determinations were made on these. The fat free dry bones were then finely ground and samples weighed out of this material for moisture, fat, ash, phosphorus, and nitrogen determinations. The fat extraction was repeated, as complete extraction was not possible with the coarse material.

Moisture and Fat.

Each sample was weighed out and placed in a glass extraction tube which had been prepared as follows. The tube was stuffed rather firmly with absorbent cotton, and heated until warm in the electric oven. The tube was then transferred to a vacuum desiccator containing concentrated sulphuric acid as a drying agent, and the desiccator was exhausted as completely as possible with an air pump. In about a week the tube was weighed with the following precautions. A weighing bottle was heated in the electric oven to a temperature of approximately 100° C., in order to drive out all moisture. Another weighing bottle was counterbalanced on the balances. Air was then slowly admitted, through sulphuric acid, into the desiccator. The lid of the desiccator was removed, and the extraction tube was transferred to the weighing bottle as quickly as possible. The weighing bottle should be warm enough not to

condense any moisture but not so warm that the tube will be heated enough to disturb the balances. The weighing bottle was then transferred to a desiccator as a number of these determinations were made at once, and the tube was thus kept dry while the other tubes were being weighed. The extraction tube was taken from the desiccator, transferred rapidly to the counterbalanced weighing bottle, and the weight secured as quickly as possible. The object of these precautions of course is to offer the least possible opportunity for the absorption of moisture. If kept dry, the extraction tubes may be preserved almost indefinitely and will not need reweighing before use.

The tubes for these analyses had been previously prepared and when the material for analysis was ready, each was prepared as follows. The greater part of the cotton was removed from the tube and spread upon a piece of smooth clean glass. A samples of one or two grams was then weighed out, placed on this cotton and thoroughly incorporated in it, taking care to get as little of the sample as possible on the glass or fingers. The cotton and material was pushed back into the extraction tube again. A small portion of the cotton which had been reserved for this purpose, was used to collect any portion of the sample which may have stuck to the glass or to the fingers, or any instrument used. A drop or two of water may assist in this. The extraction tube was then placed in the vacuum desiccator

again, and extracted as previously described. The drying over sulphuric acid was continued until approximately constant weight was obtained. All weighings were made with the precautions before described. The weight of the tube was already known. The difference in these weights gave the weight of the dry sample. The fresh sample had been weighed out, so the per cent of moisture was calculated from these two weights.

The procedure for placing the blood in the tubes was somewhat different. The blood was placed in a beaker, and weighed. A small part of the cotton was taken from the tube, and some of the blood poured in on the cotton, and the cotton removed was replaced. The weight of the blood was obtained by reweighing the beaker. Care must be taken not to put in too much blood, or some will run out of the tube.

The same sample and tube was used for the fat determinations. After the sample had been dried to constant weight in the vacuum desiccator, it was extracted with anhydrous ether in a Soxhlet fat extractor. All soluble material was called fat. The same precautions were observed in weighing as were described for the moisture determination.

Moisture.

	Net weight of Sample, grams	Weight of Moisture	Per Cent Moisture	Average Per Cent Moisture
Blood	10.0045	9.1050	91.009	
Serum and	9.5601	8.6696	90.685	90.847
Water	14.7632	lost	lost	
	3.7740	3.1642	83.842	
Liver	2.6646	2.2156	83.149	83.877
	2.9199	2.4714	84.640	
	4.5737	3.6485	79.771	
Nervous	2.5783	2.0585	79.838	79.915
System	2.8943	2.3194	80.137	
	2.9005	2.2971	79.197	
Internal	2.0188	1.5899	78.754	79.571
Organs	1.7756	1.4340	80.761	
	1.5005	1.0334	68.870	
Hide	2.6086	1.7762	68.090	69.121
	3.1541	2.2206	70.404	
	60.2006	38.1300	63.338	
Skeleton	63.9471	39.9890	62.535	62.858
	61.4848	38.5520	62.702	
	2.5751	2.0010	77.706	
Flesh	2.3426	1.8020	76.923	77.818
	2.2257	1.7544	78.825	
	2.0856	0.5328	25.547	
Fat from	2.1400	0.5470	25.561	25.644
left Kidney	1.2000	0.3099	25.825	
	2.0995	1.2945	61.657	
Excreta	2.3805	1.4418	60.571	61.158
	3.0911	1.8932	61.247	
	1.6726	1.2684	75.834	
Composite	2.3876	1.7878	74.879	76.621
	1.3438	1.0636	79.149	

Fat.

	Net weight of Sample grams	Weight of Fat	Per Cent of Fat	Average Per Cent Fat.
Blood	10.0045	0.0083	0.083	0.190
Serun	9.5601	0.0401	0.419	
and Water	14.7632	0.0101	0.068	
	3.7740	0.0344	0.911	
Liver	2.6646	0.0399	1.497	1.074
	2.9199	0.0238	0.815	
	4.5737	0.3495	7.642	
Nervous	2.5783	0.1838	7.129	7.333
System	2.8943	0.2092	7.228	
	2.9005	0.1661	5.727	
Internal	2.0188	0.1392	6.895	5.790
Organs	1.7756	0.0843	4.748	
	1.5005	0.0114	0.760	
Hide	2.6086	0.0533	2.043	1.191
	3.1541	0.0243	0.770	
	60.2006	1.0511	1.746	2.437
Skeleton	63.9471	1.8427	2.882	Reestract- .855
	61.4848	1.7035	2.682	ion Total 3.290
	2.5751	0.1202	4.668	
Flesh	2.3426	0.1197	5.110	4.416
	2.2257	0.0772	3.469	
	2.0856	1.4451	69.289	
Fat from	2.1400	1.4736	68.860	68.883
left Kidney	1.2000	0.8322	69.350	
	2.0995	0.1732	8.250	
Excreta	2.3805	0.2068	8.687	8.417
	3.0911	0.2570	8.314	
	1.6726	0.0537	3.211	
Composite	2.3876	0.1042	4.364	3.185
	1.3438	0.0266	1.979	

Ash and Phosphorus.

The samples for ash and phosphorus were heated in porcelain crucibles at a faint red heat until constant weights were obtained. Some care is necessary in this ignition, as the organic matter tends to froth and run over the crucibles before it is completely carbonized. After this stage is reached, the low heat should be still preserved, especially until all the carbon is oxidized. If the proper temperature is maintained, the ash will present a honeycombed appearance. If the temperature is too high, however, the ash will fuse into a glazed mass, and may occlude carbon; also some of the salts may be volatilized.

After the weight of ash was obtained, the phosphorus in each sample was determined as follows. The crucible and ash content was placed in a 100 cc. beaker; 20 cc. concentrated nitric acid and 10 cc. concentrated hydrochloric acid were added. The beaker was then heated on the hot plate until all hydrochloric acid had been driven off and the solution was clear. This was then filtered into a 250 cc. beaker, neutralized with ammonium hydroxide and again made slightly acid with nitric acid. After adding 15 grams of ammonium nitrate, the beaker was placed on a water bath, and the temperature raised to 65°. An excess of ammonium molybdate solution was then added and allowed to stand one hour at 65°. This was then filtered, and the residue washed by decantation and on the filter

with a ten per cent solution of ammonium nitrate.

The beaker containing the filtrate was replaced by the beaker in which the original precipitation was made and the precipitate was dissolved and washed into the beaker with ammonium hydroxide and hot water. The solution was made slightly acid with hydrochloric acid and again made slightly alkaline with ammonium hydroxide. After cooling, the phosphorus was precipitated with magnesia mixture. In fifteen minutes 12 cc. of strong ammonia were added. After standing over night, the precipitate was collected on an ashless filter paper, washed with dilute ammonium hydroxide and transferred to a porcelain crucible. After drying it was ignited and weighed as $Mg_2P_2O_7$. The phosphorus is calculated as P_2O_5.

Ash.

	Net Weight of Sample, grams	Weight of Ash, grams.	Per Cent of Ash	Average Per Cent of Ash.
Blood Serum and Water	11.7798	0.0522	0.443	
	11.8469	0.0484	0.409	0.427
	11.9134	0.0510	0.428	
Liver	10.8808	0.1024	0.941	
	10.3156	0.0974	0.944	0.957
	10.6648	0.1053	0.987	
Nervous System	11.2035	0.1361	1.215	
	9.0914	0.1130	1.243	1.234
	10.7521	0.1339	1.245	
Internal Organs	11.9821	0.1064	0.888	
	12.3685	0.1129	0.913	0.902
	11.4888	0.1039	0.904	
Hide	10.6442	0.0887	0.833	
	10.2025	0.0788	0.772	0.787
	11.9656	0.0903	0.755	
Skeleton	1.0000	0.3851	38.51	On Dry Basis 38.450
	1.0000	0.3830	38.300	
	1.0000	0.3854	38.540	
				Fresh 14.281
Flesh	14.2776	0.1269	0.889	
	10.8655	0.1018	0.937	0.904
	11.9311	0.1056	0.885	
Excreta	9.5567	0.1368	1.431	
	9.1792	0.1304	1.421	1.426
	10.4350	0.1489	1.427	
Composite	12.5412	0.4235	3.377	
	12.0454	0.4090	3.395	3.554
	11.1112	0.4322	3.890	

Phosphorus.

	Net Weight of Sample	Weight of $Mg_2P_2O_7$ grams	Weight of P_2O_5 grams	Per Cent P_2O_5		Average Per Cent P_2O_5
Blood Serum and Water	11.7798	0.0101	0.00644	0.0547		
	11.8469	0.0103	0.00657	0.0555		0.0553
	11.9134	0.0104	0.00663	0.0557		
Liver	10.8808	0.0523	0.03336	0.307		
	10.3156	0.0500	0.03190	0.309		0.309
	10.6648	0.0522	0.03330	0.312		
Nervous System	11.2035	0.1096	0.06991	0.624		
	9.0914	0.0921	0.05875	0.646		0.636
	10.7523	0.1075	0.06857	0.638		
Internal Organs	11.9821	0.0620	0.03955	0.330		
	12.3685	0.0630	0.04019	0.325		0.329
	11.4888	0.0600	0.03827	0.333		
Hide	10.6442	0.0268	0.01710	0.161		
	10.2025	0.0255	0.01627	0.159		0.156
	11.9656	0.0278	0.01773	0.148		
Skeleton	1.0000	0.1076	0.068638	15.235	On	
	1.0000	0.1061	0.067681	15.040	Dry	15.223
	1.0000	0.1069	0.068192	15.153	Basis	
					Fresh	5.854
Flesh	14.2776	0.0712	0.04542	0.318		
	10.8655	0.0786	0.05014	0.461		0.373
	11.9311	0.0637	0.04063	0.341		
Excreta	9.5567	0.0082	0.00523	0.055		
	9.1792	0.0082	0.00523	0.057		0.058
	10.4350	0.0101	0.00644	0.062		
Composite	12.5412	0.1632	0.10411	0.830		
	12.0454	0.1547	0.09868	0.819		0.848
	11.1112	0.1561	0.09958	0.896		

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Nitrogen and Protein.

The nitrogen determinations were made in the usual way. The sample was placed in a 500 cc. Kjeldahl flask,

25 cc. concentrated sulphuric acid added, and approximately 0.65 grams metallic mercury. This was heated until danger of foaming was past, then about seven grams of potassium sulphate were added and the heating continued until the liquid was clear and colorless. The flask was allowed to stand until cool, then water, a little paraffine and a few pieces of granulated zinc were added. At this point 80 cc. of concentrated sodium hydroxide solution, containing potassium sulphide were added and the flask was connected with the condenser. After shaking the flask to secure thorough mixing, it was distilled over into a receiving flask containing standard hydrochloric acid. After all the ammonia had been driven over, the receiver was disconnected and titrated back against standard ammonium hydroxide, using Congo Red as an indicator. From these results the amount of nitrogen in each sample could be calculated. The protein was obtained by multiplying the nitrogen with the protein factor, 6.25.

Nitrogen and Protein.

	Net weight of Sample, grams	Net cc.of HCl used	Weight of Nitrogen	Per Cent Nitrogen	Average Per Cent Nitrogen	Average Per Cent Protein
Blood Serum and Water	1.5951	16.86	0.022896	1.435	1.424	8.900
	1.6322	16.73	0.022719	1.392		
	1.6136	17.17	0.023317	1.445		
Liver	1.6915	28.04	0.038078	2.251	2.245	14.031
	1.6870	27.86	0.037834	2.243		
	2.1264	35.08	0.047639	2.240		
Nervous System	2.5314	30.33	0.041188	1.627	1.668	10.425
	2.6339	33.23	0.045126	1.713		
	2.9650	36.32	0.049323	1.664		
Internal Organs	1.4153	22.67	0.030786	2.175	2.199	13.744
	1.7761	28.17	0.038255	2.154		
	1.3362	22.32	0.030311	2.268		
Hide	1.4035	51.96	0.070562	5.028	5.070	31.688
	1.5294	56.99	0.077392	5.060		
	1.6520	62.30	0.084603	5.121		
Skeleton dry	0.5000	28.48	0.038675	7.735	7.761 On Dry Basis	48.506
	0.5000	28.75	0.039042	7.808	2.881 On Fresh Basis	18.016
	0.5000	28.50	0.038703	7.740		
Flesh	3.1551	62.98	0.085527	2.711	2.702	16.888
	2.9084	57.91	0.078642	2.704		
	1.7919	35.51	0.048223	2.691		
Fat from left Kidney	1.0691	6.11	0.008297	0.776	0.775	4.844
	1.2036	6.88	0.009343	0.777		
	1.6735	9.54	0.012955	0.774		
Excreta	1.7933	36.66	0.049784	2.776	2.779	17.369
	2.2882	47.29	0.064220	2.807		
	1.6148	32.74	0.044461	2.753		
Composite	1.0772	22.62	0.030718	2.852	2.793	17.456
	1.1753	23.80	0.032320	2.750		
	1.2362	25.28	0.034330	2.777		

Summation of Analyses of Fresh Material.

	% Moisture	% Fat	% Ash	% P_2O_5	% Nitrogen	% Protein
Blood serum and water	90.847	0.190	0.427	0.055	1.424	8.900
Liver	83.877	1.074	0.957	0.309	2.245	14.031
Nervous System	79.915	7.333	1.234	0.636	1.668	10.425
Internal Organs	79.571	5.790	0.902	0.329	2.199	13.744
Hide	69.121	1.191	0.787	0.156	5.070	31.688
Skeleton	62. 858	3.226	14.274	5.655	2.881	18.016
Flesh	77.818	4.416	0.904	0.373	2.702	16.888
Fat from left Kidney	25.644	68.883	—	—	0.775	4.844
Excreta	61.158	8.417	1.426	0.058	2.779	17.369
Composite	76.621	3.185	3.554	~~0.848~~ 1.367	2.793	17.456

The results in the above table are obvious, and need little comment. It will be noted, however, that the per cent of fat in the blood is very low, though as a matter of fact, the result given here is too high. One of the tubes was accidentally heated too strongly when drying after the ether extraction, and gave a result much higher than the others. It is probable that a result less than 0.1 per cent would be more correct and it is doubtful if any considerable part of even that loss was fat.

Total Sulphur.

For the estimation of total sulphur and chlorine, large samples were weighed out in porcelain crucibles, dried, powdered, and parts of this were then weighed out for analysis. In estimating sulphur, the Osborne method[14] was used. Ten grams of sodium peroxide were placed in a 250 cc. nickel crucible and water slowly added until all reaction was completed. The crucible was then heated until all water was expelled as was indicated by the formation of a scum, especially at the edges. After cooling to a pasty consistency, the powdered sample was rapidly added, and thoroughly mixed with a platinum stirring rod. Heat was then carefully applied until there was little tendency to froth; this operation required twenty or thirty minutes. Sodium peroxide was than added in small quantities at a time. Whenever this was followed by flashing or by a flame, the additions were discontinued for a while. Considerable care was necessary in this operation. Thirty minutes or longer were required from the time the sodium peroxide was first added until the additions were followed by no reaction. When this point was reached, the mass was strongly heated (to a red heat) and allowed to cool. The crucible was placed in a large beaker, covered with a watch glass, and hot water carefully added to the beaker. The contents of the beaker were acidified with hydrochloric acid and after solution was complete, the liquid was boiled to drive off

excess chlorine. The solution was then filtered hot and the filter washed until free from chlorides.

The filtrate was then neutralized with ammonium hydroxide, made acid again with 4 cc. hydrochloric acid and evaporated to 400 cc.. An excess of hot ten per cent barium chloride was then added to the boiling solution, kept at the boiling point for thirty minutes and then allowed to stand over night. The precipitate was then filtered onto an ashless filter paper, washed first with dilute hydrochloric acid, then with hot water until free from chlorides. The precipitate was then dried, ignited and weighed as barium sulphate. The results are calculated to per cent of sulphur.

Total Sulphur.

Calculated Weight of Fresh Substance	Weight of Barium Sulphate	Weight of Sulphur	Per Cent of Sulphur	Average Per Cent Sulphur
8.7310 grams	0.0857	0.0117683	0.135	
47.2472 "	0.4585	0.0629612	0.133	0.149
46.3181 "	0.6080	0.0834906	0.180	

Chlorine.

A hard glass tube 40 cm. long and 15 mm. in diameter was sealed at one end, in preparation for the determination of chlorine. The thoroughly pulverized sample was then mixed with five times its weight of powdered calcium oxide. A little calcium oxide was then placed in the glass tube, the sample was transferred on top of this and then

more calcium oxide was added. The tube was clamped in an inclined position and ignited, beginning with a low temperature. This temperature was gradually raised to the highest heat of the blast lamp. The heating was begun at the lower end of the tube and gradually extended to the upper end. When carbonization was complete, the tube was cooled, the contents transferred to a beaker, and hot water added. The substance in the beaker was thoroughly washed, by decantation and on the filter with hot water. The filtrate was acidified with nitric acid and evaporated to about 300 cc. The beaker was wrapped in black paper and after cooling, an excess of a ten per cent silver nitrate solution was slowly added. The solution was now heated to incipient boiling, with occasional stirring, until the precipitate had coagulated and settled promptly after agitation. The precipitate was then filtered through a Gooch crucible, washed with hot water, and dried at 130°C. The per cent of chlorine was calculated from the weight of silver chloride.

Total Chlorine.

Calculated Weight of Fresh Substance	Weight of Silver Chloride	Weight of Chlorine	Per Cent of Chlorine	Average Per Cent Chlorine
7.0310	0.0406	0.010038	0.143	
6.7100	0.0452	0.011175	0.167	0.169
6.4860	0.0515	0.012733	0.196	

Method of Ignition for Complete Ash Analysis.

For the complete mineral analysis, 3600 grams of the composite were weighed out and dried in casseroles. Previous experience in the laboratory had shown that such material could not be ignited in platinum without serious injury to the containers, so platinum wire baskets were substituted for platinum dishes. These baskets are six or seven centimeters in diameter, of the same depth and have eight or ten ribs which meet at a common point in the bottom. The material to be ignited was placed in these baskets and heated with the naked flame. Porcelain casseroles were placed under the baskets to catch the ash and any unignited portions that might fall. The latter were returned to the baskets and the heating continued until the material was completely carbonized and nearly all the carbon was oxidized. Complete oxidation in the baskets was not possible, so the material was then transferred to platinum dishes. Hot water was added and the dishes were placed on the water bath. At frequent intervals for two or three days the liquid was decanted off, through ashless filter papers and water added again. All filtrates were preserved. Later the water was changed less frequently. After each of the last three decantations, the material was ground, thoroughly mixed, dried and ignited.

Before the last ignition, all the insoluble ash was put in one dish and the dish and contents weighed and later the weight of the dish alone was secured.

Total Water-Insoluble Ash.

Weight of dish and contents	142.8787 grams
Weight of dish	39.4591 "
Weight of insoluble ash	103.4196 "

The solution of soluble ash was evaporated in platinum dishes to about 800 cc., transferred to a 1000 cc. graduated flask and made up to volume. Aliquots were drawn from this for determinations of the soluble ash. The ash analyses, with a few exceptions, were made in duplicate.

Total Water-Soluble Ash

Two samples of 10 cc. each were drawn and placed in platinum dishes, the weights of which had previously been secured. The solution was evaporated to dryness and heated for a short time just below a red heat. The heating and weighing was continued until constant weights were obtained.

Total Water-Soluble Ash.

Aliquot	Weight of Solids	Total Weight of soluble Ash	Average
1/100	0.2090 gfams	20.90 grams	20.89 grams
1/100	0.2088 grams	20.88 grams	

ANALYSIS OF SOLUBLE ASH.

Sodium and Potassium.

The sample used for determining total solids was used for this determination also. The residue was dissolved

in 40 cc. hot water, an excess of a saturated solution of barium hydroxide added, and then the solution was left on the water bath a few minutes. The precipitate was then filtered out and washed with hot water. A few drops of ammonium hydroxide were added to the filtrate and an excess of ammonium carbonate; after standing for some time on the water bath, the precipitate was filtered out and washed with hot water. The filtrate was again evaporated to dryness on the water bath, heated for four hours in the electric oven at 100° C., and then heated to a very faint redness to expel ammonium salts. The residue was again taken up in hot water; a few drops of ammonium hydroxide, a small amount of ammonium carbonate, and a small crystal of ammonium oxalate, were added. After standing a few minutes on the water bath, the solution was filtered, evaporated on the water bath and the ammonium salts expelled with the usual precautions. The residue was again dissolved in hot water, filtered into a weighed platinum dish, a few drops of concentrated hydrochloric acid added, then evaporated and ignited with the precautions mentioned above. The dish and contents were weighed, and the gain in weight taken as the weight of the sodium and potassium chloride.

Total Chlorides.

Aliquot	Weight of Sample	Weight of KCl plus NaCl	% of Alkali Chlorides	Average % Alkali Chlorides
1/100	0.2089 Gr.	0.1902 grams	91.048	91.144
1/100	0.2089 "	0.1907 "	91.239	

The potassium determination was made directly on

the mixture of chlorides; this was dissolved in 60 cc. hot water and a few drops of hydrochloric acid were added. An excess of platinum solution was added, to convert the potassium and sodium salts into the respective chloroplatinates. This solution was then evaporated on the water bath to a thick paste, removed and treated with 80 per cent alcohol. After standing in a cool place for two hours, the precipitate of potassium chloroplatinate was filtered onto a Gooch crucible and thoroughly washed with 80 per cent alcohol. This was followed by repeated washings with ammonium chloride solution, saturated with potassium chloroplatinate, and then with 80 per cent alcohol again. The crucible was dried in a hot air oven at 110° C., and weighed.

Potassium.

Aliquot	Weight of Sample	Weight of K_2PtCl_6	Weight KCl	Weight of K_2O	% K_2O	Average % K_2O
1/100	0.2089	0.3266	0.1002	0.063282	30.293	30.418
1/100	0.2089	0.3293	0.1010	0.063805	30.543	

Sodium.

Aliquot	Weight of Sample	Weight Total Chlorides	Weight KCl	Weight NaCl	Weight Na_2O	% Na_2O	Average Na_2O
1/100	0.2089	0.1907	0.1002	0.0905	0.0480	22.978	22.811
1/100	0.2089	0.1902	0.1010	0.0892	0.0473	22.643	

Chlorine.

For this determination 10 cc. samples were drawn and each treated as follows. The sample was transferred to a 250 cc. beaker, diluted to 100 cc., and made slightly acid

with nitric acid. The beaker had been previously wrapped with black paper in order to exclude the light. A 10 per cent silver nitrate solution was added in slight excess, drop by drop. The beakers were then heated on the hot plate, to the boiling point, stirring occasionally, until the precipitate coagulated and settled readily, leaving a clear supernatant liquid. In the meantime Gooch filters were prepared, dried and weighed. The silver chloride precipitate was then transferred to the filter, thoroughly washed with hot water, dried at 120° C. and weighed.

Chlorine.

Aliquot	Weight of Sample	Weight of AgCl	Weight of Cl	Per Cent of Cl	Average Per Cent of Cl
1/100	0.2089	0.1128	0.027904	13.358	13.328
1/100	0.2089	0.1123	0.027781	13.299	

Carbon Dioxide.

The carbon dioxide was determined by the use of a Schroedter's alkalimeter. A 100 cc. sample was evaporated until it could be conveniently held by the alkalimeter and then transferred to the alkalimeter. After the alkalimeter had cooled to the room temperature, it was weighed. The hydrochloric acid it contained was then slowly admitted after which it was heated to incipient boiling and this temperature maintained for ten minutes, then cooled to room temperature again. Air was drawn through the apparatus to displace the carbon dioxide and the alkalimeter was again weighed.

Carbon Dioxide.

Aliquot	Weight of Sample	Weight of CO_2	Per Cent of CO_2	Average Per Cent CO_2
1/10	2.0890	0.0931	4.457	4.459
1/10	2.0890	0.0932	4.461	

Silica.

A sample of 200 cc. was placed in a platinum dish, evaporated on a water bath almost to dryness, hydrochloric acid added and the evaporation completed. The dish was transferred to an oven and heated at 150° C for three hours. The sample was then moistened with water, acidified with hydrochloric acid and again dehydrated for three hours. The soluble residue was dissolved in 100 cc. very dilute hydrochloric acid, filtered through an ashless filter paper and thoroughly washed, but with the smallest amount of wash water possible. All filtrates and wash waters were retained for sulphur and phosphorus determinations. The filter paper and contents was transferred to a platinum crucible, ignited and weighed.

Silica.

Aliquot	Weight of Sample grams	Weight of SiO_2	Per Cent of SiO_2	Average Per SiO_2
1/5	4.1780	0.0095 grams	0.227	0.237
1/5	4.1780	0.0103 grams	0.247	

Sulphur.

The filtrate from the silica determination was made up to the original volume, 200 cc., in a graduated

flask and aliquots were drawn from this for the determination of phosphoric acid and sulphuric anhydride. A 10 cc. sample was drawn, transferred to a 400 cc. beaker and diluted to 200 cc. The solution was made distinctly acid with hydrochloric acid, heated to boiling and an excess of 10 per cent solution of hot barium chloride was added slowly with constant stirring. A temperature almost boiling was maintained for thirty minutes and then the beaker was allowed to stand over night. The precipitate was washed by decantation and on the ashless filter paper with hot water. It was then transferred to a platinum crucible, ignited and weighed as barium sulphate.

Sulphur.

Aliquot	Weight of Sample	Weight of $BaSO_4$	Weight of SO_3	Per Cent of SO_3	Average Per Cent SO_3
1/100	0.2089	0.1127	0.038656	18.505	18.530
1/100	0.2089	0.1130	0.038759	18.554	

Phosphorus.

A sample of 10 cc. was measured out into a 250 cc. beaker and diluted to 50 cc. This was made slightly acid with nitric acid and 15 grams of dry ammonium nitrate were added. The solution was warmed to 65° C., and 50 cc. molybdate solution added. The temperature was maintained for one hour, then the yellow precipitate was filtered off and washed with 10 per cent ammonium nitrate solution, made slightly acid with nitric acid. The filtrate was replaced by the beaker in which the precipitation was made and the precipitate washed into this beaker with dilute ammonium hydroxide and hot water. This solution was made slightly

acid with hydrochloric acid, then faintly alkaline with ammonium hydroxide. After cooling, 12 cc. of magnesia mixture were slowly added, with constant stirring; in fifteen minutes one-third the volume of ammonium hydroxide was added and allowed to stand over night. The precipitate of magnesium ammonium phosphate was then filtered off and washed with dilute ammonium hydroxide. The filtrate was replaced by the beaker in which the original precipitation was made and the precipitate was dissolved with dilute hydrochloric acid. The filtrate was then washed with water until free from chlorides. A few drops of magnesia mixture were added and the solution made slightly alkaline with ammonium hydroxide. After stirring two or three minutes this was allowed to stand fifteen minutes, then one-third the volume of ammonia water was added and allowed to stand five hours. The magnesium ammonium phosphate was filtered off, washed with dilute ammonium hydroxide, dried and ignited to whiteness.

Phosphorus.

Aliquot	Weight of Sample	Weight of $Mg_2P_2O_7$	Weight of P_2O_5	Per Cent P_2O_5	Average Per P_2O_5
1/100	0.2089	0.0435	0.027749	13.283	13.235
1/100	0.2089	0.0433	0.027621	13.222	

Summation of Water-Soluble Ash.

	Weight of Constituents in Soluble Ash	Percentage Composition in Soluble Ash
K_2O	6.3545 grams	30.419
Na_2O	4.7652 "	22.811
Cl	2.7842 "	13.328
CO_2	0.9314 "	4.459
SiO_2	0.0495 "	0.237
SO_3	3.8709 "	18.530
P_2O_5	2.7685 "	13.253
Sum	21.5242 "	103.037
Deduct O=Cl	0.6281 "	3.071
Remainder	20.8961 "	99.966

Insoluble Ash.

A sample of five grams was placed in a 600 cc. beaker and boiled for three hours in dilute hydrochloric acid. A small portion was insoluble and was filtered off onto an ashless filter paper. This residue was ignited in a platinum crucible and fused with sodium potassium carbonate. The fusion was dissolved in hot water and added to the filtrate. The filtrate was evaporated to dryness in a platinum dish and the residue dehydrated for the determination of silica, as described in the estimation of silica in the water soluble ash.

Silica.

When dehydration of the silica was complete, the acid soluble portion was dissolved in dilute hydrochloric acid and filtered off into a 500 cc. graduated flask. The

silica was retained on an ashless filter, ignited and weighed.

Silica.

Sample	Weight of SiO_2	Per Cent of SiO_2	Average Per Cent SiO_2
5 grams	0.0151	0.302	0.316
5 "	0.0165	0.330	

The filtrate was made up to volume in the 500 cc. flask and aliquots drawn for the determination of phosphorus, iron, calcium and magnesium.

Phosphorus.

A 20 cc. sample was drawn into a 250 cc. beaker and an equal volume of strong nitric acid added. This was left on the hot plate until all the hydrochloric acid had been driven off. The solution was made alkaline with ammonium hydroxide, slightly acid again with nitric acid, 15 grams of dry ammonium nitrate added and warmed to 65° C. The procedure for this determination was the same as for the phosphorus in the water soluble ash.

Phosphorus

Aliquot	Weight of Sample	Weight of $Mg_2P_2O_7$	Weight of P_2O_5	Per Cent P_2O_5	Average Per P_2O_5
1/25	0.2 grams	0.1362	0.086882	43.441	43.457
1/25	0.2 "	0.1363	0.086946	43.473	

Iron.

Samples of 10 cc. each were drawn and transferred to 250 cc. Erlenmeyer flasks. To this 1 cc. concentrated sulphuric acid was added and evaporated on the hot plate to the appearance of white fumes. After cooling, 25 cc. water,

and 0.5 grams of zinc were added, the flasks were equipped with reductors and heating continued on the hot plate until all the zinc was dissolved. The solution was then titrated with a standard solution of potassium permanganate.

Iron.

Aliquot	Weight of Sample	cc. $KMnO_4$	factor of Fe_2O_3	% Fe_2O_3	Average % Fe_2O_3
1/50	0.1 gram	1.37	0.0008147894	1.116	
1/50	0.1 "	1.40	"	1.141	
1/50	0.1 "	1.44	"	1.172	1.160
1/50	0.1 "	1.43	"	1.165	
1/50	0.1 "	1.45	"	1.181	
1/50	0.1 "	1.46	"	1.189	

Calcium.

A 50 cc. sample was drawn into a 350 cc. beaker, neutralized with a slight excess of ammonium hydroxide and two grams of ammonium acetate were added. A large excess of strong acetic acid was added and allowed to stand one hour. The solution was filtered and the precipitate on the filter paper was washed with dilute acetic acid. The filtrate was set aside, replaced by the beaker containing the original precipitate and the precipitate was dissolved and washed into this beaker with hot dilute hydrochloric acid; the filter paper was made alkaline with ammonium hydroxide. The hydrochloric acid solution was again neutralized with ammonium hydroxide, acidified with acetic acid, filtered and washed. The filtrates were combined and the precipitate rejected.

The combined filtrates were heated to the boiling point and a hot solution of ammonium oxalate added slowly in excess. This was kept at the boiling point one hour, set aside and filtered when the supernatant liquid became clear. The filtrate was replaced by the beaker in which the original precipitation was made and the precipitate of calcium oxalate was washed into this with hot, dilute hydrochloric acid. The calcium solution was again made alkaline with ammonium hydroxide, acidified with acetic acid and a few drops of ammonium oxalate added. The precipitate was digested and filtered as before, taking care that the filter paper was alkaline again. The filtrates were combined and preserved for the determination of magnesium.

A 250 cc. flask was placed under the filter and the calcium oxalate precipitate was washed through an opening in the filter paper into the flask; not water and dilute sulphuric acid were used for this purpose. After cooling the flask was made up to volume. Aliquots were drawn, transferred to Erlenmeyer flasks, diluted to 200 cc. and titrated with potassium permanganate solution at a temperature of 75°.

Calcium.

Ali-quot	Weight of	cc. $KMnO_4$	$KMnO_4$ factor	Weight CaO	% CaO	Average CaO
1/25	0.02 gr.	34.50	.0002862222	0.0098746	49.373	
1/25	0.02 "	34.50	"	0.0098746	49.373	
1/25	0.02 "	34.51	"	0.0098774	49.387	49.370
1/25	0.02 "	34.45	"	0.0098602	49.301	
1/25	0.02 "	34.53	"	0.0098831	49.415	
1/25	0.02 "	34.50	"	0.0098746	49.373	

Magnesium.

The filtrates from the calcium determinations were evaporated until the salts in solution began to crystallize. The solution was then cooled and diluted until all salts were dissolved. Ammonium hydroxide was added in slight excess and then a solution of di-sodium phosphate was slowly added until precipitation was complete. After standing a short time, one-third the volume of ammonium hydroxide was added and the precipitate was allowed to stand over night. The clear liquid was decanted through a filter paper and the precipitate washed by decantation and on the filter paper with dilute ammonium hydroxide. The filtrate was replaced by the beaker in which the original precipitation was made and the precipitate dissolved and washed into the beaker with hot dilute hydrochloric acid and thoroughly washed with water. After adding 3 cc. di-sodium phosphate solution, the solution was made slightly ammoniacal and allowed to stand fifteen minutes. One-third the volume of ammonium hydroxide was then added and the precipitate allowed to stand several hours. The precipitate of magnesium phosphate was filtered onto an ashless filter paper, washed with dilute ammonium hydroxide and ignited in a platinum crucible. The magnesium oxide was calculated from the weight of pyrophosphate.

Magnesium.

Aliquot	Weight of Sample	Weight of $Mg_2P_2O_7$	Weight of MgO	% MgO	Average % MgO
1/10	0.5 gram	0.0286	0.010355	2.071	2.104
1/10	0.5 "	0.0295	0.010681	2.136	

Sodium and Potassium.

The J. Lawrence Smith method was used in this determination. A. 0.5 gram sample of the insoluble ash was intimately mixed in an agate mortar with an equal weight of ammonium chloride. A small amount of calcium carbonate was placed in the bottom of a platinum J? Lawrence Smith crucible. The greater part of the calcium carbonate used was then mixed with the sample in the mortar and then transferred to the crucible. A small portion of the carbonate was used to rinse out the mortar, added to the crucible, and the remainder was placed on top of this. The total amount of calcium carbonate used was 4 grams. The crucible was clamped in a sloping position and gently heated until the ammonia had been driven off. The lower part of the crucible was then heated strongly for one hour, in order that only the sample should reach the high temperatures.

After cooling, the contents of the crucible were transferred to a casserole, 75 cc. of water added and kept near the boiling point for thirty minutes. The liquid was then decanted through a filter paper, more water added and the heating continued. The contents of the casserole were then transferred to the filter and thoroughly washed with hot water. From this point the determination is carried on as described in the analysis of the soluble ash.

Total Chlorides.

Sample	KCl plus NaCl
0.5 grams	0.0301 grams
0.5 "	0.0295 "

Potassium.

Sample	Weight of K_2PtCl_6	Weight of KCl	Weight of K_2O	Per Cent K_2O	Average Per Cent K_2O
0.5 gr.	0.0187	0.00573	0.00362	0.724	0.726
0.5 "	0.0188	0.00576	0.00364	0.728	

Sodium.

Sample	Weight KCl + NaCl	Weightof KCl	Weight of NaCl	Weight of Na_2O	% Na_2O	Average % Na_2O
0.5 gr.	0.0295	0.00573	0.02437	0.01292	2,584	2.551
0.5 "	0.0301	0.00516	0.02374	0.01259	2.518	

Summation of Water+Insoluble Ash.

	Per Cent	Absolute Weight
SiO_2	0.316	0.3268 grams
P_2O_5	43.457	44.9431 "
Fe_2O_3	1.160	1.1997 "
CaO	49.370	51.0582 "
MgO	2.104	2.1759 "
Na_2O	2.551	2.6382 "
K_2O	0.726	0.7508 "
Total	99.684	103.0927 "

Summation of Total Ash.

Per Cent Constituents in Terms	Soluble Ash of Total Ash	Per Cent Insoluble Ash in Terms of Total Ash	Per Cent Constituents in Total Ash.
K_2O	5.112	0.604	5.716
Na_2O	3.834	2.122	5.956
Cl	2.239	none	2.239
CO_2	0.749	none	0.749
SiO_2	0.039	0.264	0.303
SO_3	3.114	none	3.114
P_2O_5	2.227	36.235	38.462
Fe_2O_3	none	0.966	0.966
CaO	none	41.073	41.073
MgO	none	1.750	1.750
Total	17.314	82.964	100.328
O=Cl	0.516		0.516
	16.798		99.812

Constituents	Per Cent Constituents in Body Substance	Gross Weight Constituents in Body
K_2O	0.2031	66.4685
Na_2O	0.2117	69.2831
Cl	0.0796	26.0507
CO_2	0.0266	8.7054
SiO_2	0.0108	3.5345
SO_3	0.1107	36.2388
P_2O_5	1.3669	447.3454
Fe_2O_3	0.0343	11.2264
CaO	1.4597	477.7160
MgO	0.0622	20.3562
Total	3.5656	1166.9150
Deduct O=Cl	.0179	5.8772
	3.5477	1161.0378

Loss of Sulphur and Chlorine by Ignition.

The per cent of sulphur as given on page 30 is 0.149. Calculating on this basis, the body of the calf would contain in various conbinations 236.0160 grams of sulphur. According to the amount of sulphuric anhydride reported in the ash, the body would only contain 76.5123 grams of sulphur. This would indicate a loss of 67.582 per cent of the sulphur by the method of ignition used.

The per cent of chlorine as given in the body organism on page 31 is 0.169. The body of the calf then would contain 267.696 grams of combined chlorine. According to the ash analysis the calf body contains 126.0864 grams, indicating a loss of 52.896 per cent of the chlorine by this method of ignition.

Grams Sulphur in Calf Body	Grams Sulphur in Body according to ash analysis	Loss of Sulphur in Ignition	Per Cent Loss of Sulphur in Ignition
236.0160	76.5123	159.5037	67.582

Grams Chlorine in Calf Body	Grams Chlorine in Body according to ash analysis	Loss of Chlorine in Ignition	Per Cent Loss in Ignition
267.6960	126.0864	141.6096	52.896

The amount of loss depends on conditions evidently and is not constant, as Mr. Vanatta, of this station, last year found a greater loss than that reported here, especially in sulphur. He gives a number of references concerning this loss and concerning methods of making the analysis but it is not considered necessary to repeat them here.

	Reported in This Investigation	Reported by Mr. Vanatta
Per Cent Loss of Sulphur	67.582	91.08
Per Cent Loss of Chlorine	52.896	62.24

Comparison.

A calf embryo, one hundred eight-five days old, was analyzed in this laboratory in 1909, although a complete examination of the ash was not made. A comparison of results with those obtained in this investigation would have especial value as the animals were of the same species and the methods of analysis were similar. For further comparison, similar work by other investigators might well be included also. The results given here for this purpose are an average of Wilson's results on page 11, Brubacher's on page 8 and Camerer's on page 4. The results of Wilson here are calculated on a body weight basis.

	New Born Calf	Calf Foetus 185 days	New Born Pig Wilson	New Born Infant Camerer	Human Foetus 28 wks. Brubacher	Human Foetus 36 Wks. Brubacher
Moisture %	76.21	84.80	80.03	71.7	80.75	75.28
Fat %	3.18	3.36	1.48	12.8	3.95	8.42
Protein %	17.46	10.46	12.24	11.5		
Ash %	3.55	1.78		3.6	3.00	3.12
P_2O_5 %	0.85	0.28			1.09	1.15
CaO %			1.78		1.04	1.13

From a consideration of the first two columns, it seems that the per cent moisture is higher in the earlier stages

of the embryonic period. In the cases reported above, there is not much difference in the protein, if the calculations are made on a dry basis. On the same basis, however, the per cent of ash is the higher in the new born calf, while the per cent of phosphorus is considerably higher. The most striking comparison, however, is that of the relative weights of the two specimens. Although this foetus had been carried one hundred eighty-five days, yet its weight was about one-fifth that of the new born calf. Comparing the weights of dry matter in the two cases, the weight of the foetus is but little over one-eighth of the other. This indicates that the energy of growth is much greater in the last three months than in the preceding part of the prenatal period.

The analyses given here are not much different from what would be expected after a comparison with other work, but perhaps some of the results are worthy of comment. The high per cent of fat reported in one case by Camerer, in another by Brubacher, is rather unusual. The phosphorus reported by Brubacher is also unusually high.

Ash Analyses.

A consideration of ash analyses might also have a certain interest, especially that of a new born calf analyzed by Mr. Vanatta. Others given are of a new born infants; by Hougounenq on page 2, by Camerer on page 3, and by de Lange on page 4; also of a new born guinea-pig on page 5. The result of Camerer is the average of two analyses.

Constituents	New Born Calf. Hogan	New Born Calf. Vanatta	New Born Infant. Hugouneng	New Born Infant. Camerer	New Born Infant. de Lange	New Born Guinea-pig. Abderhalden
K_2O	5.716	4.653	6.20	7.9	6.54	8.1565
Na_2O	5.956	6.226	8.12	9.2	8.80	6.9171
Cl	2.239	1.949	4.26	7.7	6.36	9.1119
CO_2	0.749	0.191	1.89			
SiO_2	0.303	0.639				
SO_3	3.114	1.107	1.50			
P_2O_5	38.462	39.382	35.28	38.9	37.61	42.0667
Fe_2O_3	0.966	0.983	0.39	0.9	1.69	0.2409
CaO	41.073	43.113	40.48	36.1	38.89	32.1758
MgO	1.750	2.074	1.51	0.9	1.37	3.3682
Sum	100.328	100.317	99.63	101.6	101.26	102.0371
O=Cl	0.516	0.440	0.96	1.8	1.43	2.0371
Total Ash	99.812	99.877	98.67	99.8	99.83	100.0000

A comparison of the first two columns probably has the greater value, due to similarity of conditions. The calves were of the same mother and met death in the same way; the methods of analysis also were quite similar. The specimen used in this analysis was carried a few days over time, however, and was considerably heavier than the one Mr. Vanatta analyzed.

The difference in the relative amounts of sodium and potassium is perhaps the most important feature, although the data affords no explanation. The amount of carbon

dioxide, sulphur and chlorine in the ash depends on conditions during ignition and variations are of no significance.

The results of Abderhalden are interesting in the very low per cent of iron reported. This is also the only case reproduced here in which the per cent of potash is greater than the per cent of soda. As was mentioned on page 2, Hougounenq points out that in the young of the dog, cat and rabbit, the potash ordinarily predominates. Bunge[15] also reports some results which bear out that statement.

Older Animals.

Since the composition of the new born calf has been compared, as far as possible, with that of calf embryos, a comparison with more mature animals might also be desirable. The results used for comparison here are those of Lawes and Gilbert.

Constituents	New Born Calf as Determined in this Investigation	Fat Calf	Half Fat Ox
Fe_2O_3	0.996	0.53	0.97
CaO	41.073	43.95	45.26
MgO	1.750	2.20	2.03
K_2O	5.716	5.40	4.41
Na_2O	5.956	3.82	3.08
P_2O_5	38.462	40.37	40.32
SO_3	3.114	1.08	0.86
CO_2	0.749	1.34	1.97
Cl	2.239	1.55	1.24
SiO_2	0.303	0.12	0.24
Sum	100.328	100.36	100.28
O=Cl	0.516	0.36	0.28
	99.812	100.00	100.00

The results given here may indicate that the per cent of iron is higher in the new born animal than later in life, though the high per cent in the half fat ox makes such a conclusion uncertain. However, that may be in this case. Abderhalden[13] makes that general statement, though he notes some exceptions.

The per cent calcium and phosphorus is higher in the older animal which of course is to be expected.

The higher per cent of potash may be worthy of interest though the limited data given here makes a conclusion impossible. A number of analysts, however, have worked on similar problems, notably Abderhalden. In one article[14] he states that there is no sodium in the blood corpuscles of the horse, rabbit or swine, while sodium is present in carnivorous animals and ruminants. If this fact had any effect, it would increase the per cent of sodium relative to that of potassium. On the page following the one just cited[13], Abderhalden takes up this subject from another standpoint and shows that the haemoglobin per unit body weight is greatest at birth and then declines. These facts may have nothing to do with the lower per cent reported by Lawes and Gilbert, though they afford room for some speculation.

Mother's Milk and the Young Animal.

The rélation of the mineral composition of mother's milk to that of the foetus or young, has been much discussed

for some time. Bunge[15, 18] and Abderhalden[7] have both written extensively on that subject and take the ground that the composition of mother's milk varies with the rate of growth of the young. They have also stated that the per cent of the ash constituents is parallel in the milk and in the young. Though they present evidence to support this view, yet some of the other papers referred to here, indicate that the infant at least is an exception to that fact. Bearing in mind this previous discussion, the relation between the mineral constituents of a new born calf and cow's milk may have a certain interest. Such a comparison presents certail difficulties, as the composition of milk varies considerably with individuals and with the period in lactation. The analyses given below[15, 19, 18] are considered as suitable for comparative purposes, however, as any available.

Constituents	Analysis of New Born Calf	Analysis of Cow's Milk (Schrodt)	(Bunge)
K_2O	5.716	25.42	22.14
Na_2O	5.956	10.94	13.91
Cl	2.239	14.60	21.27
CO_2	0.749		
SiO_2	0.303		
SO_3	3.114	4.11	
P_2O_5	38.462	24.11	24.75
Fe_2O_3	0.966	0.11	0.04
CaO	41.073	21.45	20.05
MgO	1.750	2.54	2.63
Sum	100.328	103.28	
O=Cl	0.516	3.28	
	99.812	100.00	

Although it may be true as Bunge and Abderhalden assert, that the more rapid the growth of the young, the higher the per cents of the various constituents in the mother's milk. The above data does not indicate that the composition of the mineral matter in the young and in the mother's milk is parallel.

SUMMARY.

An analysis can not well be summarized in words and the analysis of a single specimen does not permit of many general conclusions. It might be well, however, to note some of the facts brought out from this investigation; a comparison with the analysis of the calf foetus as previously noted, will assist in drawing general conclusions.

The percentage of moisture in the new born calf is lower than in the earlier stages of development, indicating that the percentage of solids in the embryo increases with age. The percentage of fat also increases but in a smaller proportion than the solids.

The percentage of phosphorus is much higher in the new born calf than in the earlier stages of development and is still about twice as high, even if calculated on a dry basis, indicating a rapid formation of bony tissue.

Though the percentage of ash is considerably higher in the new born calf than in the calf embryo, yet this difference disappears when calculated on a dry basis; in this case the dry matter of the foetus even contains a slightly higher per cent of ash than does the dry matter of the new born calf.

As shown by the analysis of the fresh material of the calf, the per cent of phosphorus in the ash of the blood is very low. As would be expected, the amount of

phosphorus in the ash of the nervous system is high.

The per cents of fat and protein in the excreta are relatively high.

The amount of ether-soluble material in the blood is very low, there might easily be no fat whatever present.

The mineral constituents of the young and of mother's milk are not necessarily parallel in composition.

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