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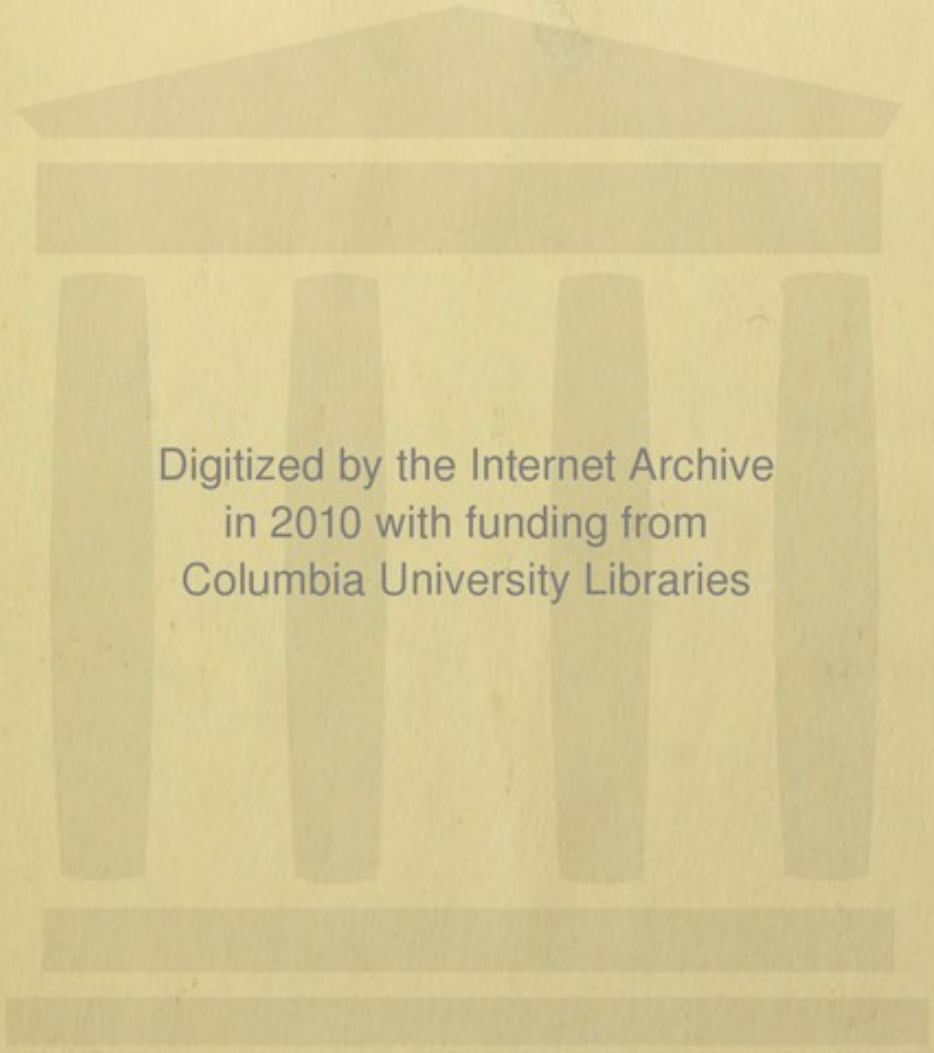

Biochemical Studies of Beryllium Sulfate.

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE OF COLUMBIA UNIVERSITY

BY

EMILY CROMWELL SEAMAN, B.S., M.A.

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ACKNOWLEDGMENT.

This research has been done at the suggestion and under the personal direction of Professor William J. Gies. During its progress he has given the investigation much personal attention. I wish to express my sincere appreciation and gratitude for his interest and assistance and especially I wish to thank him for the generosity which made it possible for me to work in his laboratory.

EMILY C. SEAMAN.

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COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK CITY.
May 16, 1912.

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feces. The raw meat was preserved in a frozen condition.[1] The cracker meal was thoroughly mixed and kept entirely dry in large glass-stoppered bottles. The best quality of lard was purchased in small amounts and kept in a refrigerator. Infusorial earth was used instead of bone-ash in some of the experiments in order to prevent possible conversion of administered beryllium into the insoluble compounds which bone-ash might induce. Distilled water was used. The dog was given all the daily portion of food at one time, the solid ingredients being thoroughly mixed with water.

Periods and Weights.—Each day of the experiment ended at 2.30 P.M. The dogs were weighed just before being fed. A new period of record was always begun with the day on which the dog was placed under new conditions.

Collection of Excreta.—The urine was collected each day just before feeding, and the daily volume recorded. The cage was washed every second day, the washings being added to the total urine for the two days and the volume made up to 2000 cc. with distilled water. The total nitrogen was determined from this. The fecal matter was removed from the cage as soon as possible after its elimination, placed in an evaporating dish and dried as speedily as possible by exposure to the air. The feces for each day were kept separate and weighed when thoroughly dry. It was noticeable that, with the exception of a condition which will be mentioned later in the experiment, the appearance of the material was uniformly dry and chalky, which agrees with the previous experiences in the use of infusorial areth in nutritional experiments directed here by Prof. Gies.

Analysis.—The total ingested nitrogen, sulfur, phosphorus and inorganic matter were determined from the food. The nitrogen of the urine was determined on the total volume for every two days and the nitrogen in the feces for each period. The ash, sulfur and phosphorus of the urine and feces were de-

[1] Gies: *American Journal of Physiology*, 1901, v, p. 235; Gies and collaborators: *Biochemical Researches*, 1903, p. 69 (Reprint No. 1).

termined for the period. The urine was preserved with thymol. The feces were finely powdered before analysis.

ANALYTICAL METHODS.—The total nitrogen was determined by the Kjeldahl process, oxidation being effected with concentrated sulphuric acid and a little cupric sulfate. The total sulfur and phosphorus of the urine were determined by the caustic alkali fusion methods. Because of the presence of silicate in the feces it was necessary to remove silica after fusion before determining the sulfur and phosphorus. This was done by dehydration with acid followed by desiccation, extraction and filtration. The total inorganic material was obtained by evaporating the sample to dryness and charring at a low redness until all the carbonaceous material had been burned and only a white residue remained.

BERYLLIUM SULFATE.—The beryllium sulfate used in the metabolism experiments was a pure product obtained from Dr. Charles L. Parsons for this special work.

FIRST METABOLISM EXPERIMENT.—The dog selected for this experiment was a healthy female bull terrier weighing about twelve kilos.

Diet.—The diet was the same throughout the experiment except that bone-ash was used during the fore and first dosage periods. This was then changed and infusorial earth used in its place for the remainder of the metabolism work, for the reason stated above (Table I).

TABLE I.

Composition of Daily Diet. Experiment I.

Ingredients.	Lean meat.	Cracker Meal.		Lard.	Bone-ash.	Infusorial earth.	Water.	Total.	
Days	1–36	1–33	34–36	1–36	1–17	18–36	1–36	1–17	18–36
	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	cc.	Grams.	Grams.
Daily amount	178.0	48.0	48.0	35.0	12.0	10.0	415.0	688.0	686.0
Nitrogen	6.96	0.84	0.93	0.0007	0.0012			7.8	7.89
Sulfur	0.338	0.07	0.078		0.02			0.43	0.41
Phosphorus	0.16	0.06	0.06		1.08			1.36	0.28
Inorganic matter	1.96	0.40	0.62		11.88	9.7		14.2	12.0

Preparatory Period.—Eight days were sufficient for the preparatory period. During this time the animal became accustomed to the food and environment. The weight became nearly constant. The preparatory period ended on Nov. 24, 1910 at 2.30 o'clock and on that day the experimental work was begun. It was continued uninterruptedly until 2.30 o'clock on December 29, 1910—(36 days). The experiment was divided into six periods of different lengths and conditions as follows:

Fore Period.—Normal conditions. Days, 1–8. Nov. 24 to Dec. 1, 1910. During this period entirely normal conditions were maintained.

First Dosage Period.—Influence of beryllium sulfate administered *per os*. Days, 9–16. Dec. 2–9, 1910. On the ninth day the first dose of beryllium sulfate was given. The dosage was started with 0.1 gram and increased each day until on the last day of this period 5.0 grams had been administered. The dose was always given just before feeding by enclosing the beryllium sulfate in a ball made of part of the weighed meat. The mass was swallowed quickly without mastication and the remainder of the food given at once. No symptoms were produced by this amount of beryllium sulfate but it was decided not to prolong this dosage period. There was a slight decrease in weight.

Special Period.—Days, 17–18. Dec. 10–11, 1910. Because of a continued loss in weight and variations in the volume of urine it was decided to consider the two days following the dosage period as a special period.

Intermediate Period. — Days, 19–24. Dec. 12–17, 1910. During this period there was a tendency toward restoration of normal nutrition. The weight still decreased but toward the end of the period it began to increase and the volume of urine struck a normal average.

Second Dosage Period. — Days, 25–28. Dec. 18–21, 1910. After an interval of eight days beryllium sulfate was again administered. As in the first dosage period the amount was started with 0.1 gram. It was increased each day until

by the fourth day, 3.6 grams of beryllium sulfate had been given. At this point marked effects were produced. Previous to the time this amount was given (2 P.M., Dec. 21) there were no signs of sickness. At 9 A.M. the next morning (Dec. 22) a large amount of vomit consisting of undigested meat and mucus was found on the pan and in the urine. Some of the material on the pan was dry indicating that there had been an attack of vomiting sometime between midnight when the dog was last inspected and 9 A.M. There were no other signs of sickness. The dog's nose was cold and she seemed full of life. The urine containing the vomit was strained and the solid particles from this and from the pan were weighed and added to the next day's food (Dec. 22). In anticipation of vomiting the urine receiver had purposely been kept free from thymol. The volume of urine was decidedly increased, but this was probably due to the presence of the vomit. Just before the time for feeding on the same day the dog vomited twice. This vomit consisted of about 25 cc. of colorless froth with a little liquid. It was allowed to remain on the pan to be added to the urine. No more beryllium sulfate was given. The maximum amount given in this experiment was evidently a toxic dose for this dog and could not be satisfactorily exceeded in such work. The beryllium sulfate had probably exerted a deleterious effect on digestion and was possibly diuretic. The feces for this period were black and soft in marked contrast to those of the former periods.

After Period.—Days, 29–36. Dec. 22–29, 1910. Although no more beryllium sulfate was given, the dog showed a tendency to vomit on the first day of this period (Dec. 23). At 11 A.M. and again at 12 M. she ejected small quantities of thin liquid. There were no solid particles in the vomit at either time. The material was added to the day's urine. The dog was very lively, the nose was cold and she showed no other signs of sickness. During this after period there was a gradual but constant increase in weight.

Analytical Results. — The data in the daily records and

the summary of results with the daily averages for the first metabolism experiment are given in Tables II–IV.

Discussion of Results.—During the fore period the animal showed a fluctuation in weight ending in a slight gain. The period was marked by a decided plus balance of the nitrogen, ash and phosphorus and a small plus balance of the sulfur. It was evident that the diet was more than sufficient for the dog's requirements. The first dosage period was without marked effect although a total of over 5 grams of beryllium sulfate were given. The weight fluctuated slightly during the period but there was very little loss at the end. There was decrease of nitrogen and sulfur elimination but an increase in elimination of ash. The phosphorus remained practically the same. There was an increase in the volume of urine.

It seemed quite evident from the data for the two periods following (and later experiments bear this out) that the beryllium sulfate was cumulative in its influence. During this time there was a decided loss of weight and further increase in the volume of urine. The first two days were so marked in these respects that the excreta were analyzed for a separate period. The results showed a great loss in nitrogen, ash, sulfur and phosphorus suggesting that the beryllium sulfate had produced decided nutritive disturbance. During the following six days (the intermediate period) there was some return to normal conditions as was shown by the decreased elimination of nitrogen, sulfur and phosphorus.

In the second dosage period nearly 4 grams of beryllium sulfate were given in four days. It had marked toxic effect, producing vomiting. Up to this time the feces had been hard, chalky, and uniform in character, but on the third day of the period, they began to be dark and decidedly soft. This condition lasted until the second day of the after period. There was a marked loss of weight and an increased elimination of nitrogen and sulfur during this second dosage period but as in the former dosage and after periods, the effect of the beryllium sulfate extended beyond the time of

the administration. Here there was a still greater loss of body nitrogen, sulfur and phosphorus.

The result of the first experiment showed a decided loss

TABLE II. *Experiment I.*

Fore Period.

Date. Nov.	Body weight. Kilos.	$BeSO_4$. Gram.	Urine. Vol. cc.	Sp. gr.	Reaction, litmus.	Nitrogen. Grams.	Feces. Dry weight. Grams.
24	11.82	....	405	1017	acid	12.07	15.4
25	11.82	....	437	1015	acid		31.7
26	11.82	....	431	1016	am. ac.*	12.18	26.6
27	11.80	....	445	1015	acid		20.2
28	11.84	....	435	1015	acid	11.55	14.2
29	11.84	....	405	1015	acid		40.5
30	11.84	....	510	1014	acid	12.53	none
Dec. 1	11.83	....	435	1015	acid		37.0

First Beryllium Sulfate Period.

2	11.83	0.1165	470	1015	am. ac.	11.22	5.5
3	11.76	0.2131	483	1014	am. ac.		36.0
4	11.78	0.2131	470	1015	acid	12.87	lost
5	11.84	0.2581	475	1015	acid		17.3
6	11.78	0.5034	433	1014	acid	10.03	20.2
7	11.80	1.0012	506	1016	acid		16.5
8	11.79	1.2473	483	1016	acid	12.42	17.0
9	11.80	1.5002	418	1017	acid		35.0

Special Period.

10	11.79	....	480	1017	acid	16.16	13.5
11	11.69	....	550	1015	am. ac.		14.5

* Urines that were amphoteric but apparently more acid than alkalin are referred to as "am. ac." in the Tables.

TABLE II—(*Continued*).

Intermediate Period.

Date. Nov.	Body weight. Kilos.	$BeSO_4$. Grams.	Urine. Vol. cc.	Sp. gr.	Reaction, litmus.	Nitrogen. Grams.	Feces. Dry weight. Grams.
12	11.71	....	423	1016	acid	13.59	25.5
13	11.68	....	510	1016	acid		14.0
14	11.69	....	425	1015	acid	13.03	20.0
15	11.69	....	475	1017	am. ac.		11.5
16	11.73	....	470	1015	acid	12.03	14.5
17	11.70	....	445	1015	acid		17.0

Second Beryllium Sulfate Period.

18	11.75	0.1003	464	1015	am. ac.	13.32	10.5
19	11.74	0.5039	425	1016	am. ac.		23.5
20	11.77	1.0110	473	1018	am. ac.	14.27	11.5
21	11.61	2.0100	595	1016	am. ac.		18.5

After Period.

22	11.63	....	415	1020	am. ac.	15.00	21.5
23	11.68	....	446	1017	am. ac.		11.5
24	11.73	....	433	1017	am. ac.	14.21	22.0
25	11.74	....	490	1016	am. ac.		14.0
26	11.77	....	455	1019	am. ac.	14.83	19.5
27	11.77	....	455	1019	am. ac.		20.0
28	11.80	....	415	1016	am. ac.	14.27	8.0
29	11.82	....	468	1017	am. ac.		13.0

Table III.

Experiment I.—Data in grams.

Nitrogen Balance.

	Fore period, 8 days.		1st $BeSO_4$ period, 8 days.		Special period, 2 days.		Intermediate period, 6 days.		2nd $BeSO_4$ period, 4 days.		After period, 8 days.	
Nitrogen from food		62.4		62.4		15.6		46.8		30.0		60.3
Nitrogen from urine	48.33	52.9	46.54	50.0	16.16	16.98	38.65	41.40	27.59	29.09	58.31	61.21
Nitrogen from feces	4.60		3.46		0.82		2.75		1.50		2.90	
Nitrogen balance		+9.5		+12.4		—1.38		+5.4		+0.91		+0.91
Daily averages		+1.19		+ 1.55		—0.69		—0.9		+0.22		—0.12

Balance of Inorganic Matter.

Ash from food		113.92		113.92		24.0		72.0		48.0		96.9
Ash from urine	14.98	88.29	15.04	102.51	3.44	19.14	11.30	75.23	8.64	50.88	16.42	99.45
Ash from feces	73.31		87.47		15.70		63.93		42.24		83.03	
Ash balance		+25.63		+11.4		+4.86		—3.23		—2.88		—2.55
Daily average		+ 3.2		+ 1.4		+2.4		—0.54		—0.72		—0.32

TABLE III—(*Continued*).

Sulfur Balance.

	Fore period, 8 days.		1st $BeSO_4$ period, 8 days.		Special period, 2 days.		Intermediate period, 6 days.		2nd $BeSO_4$ period, 4 days.		After period, 8 days.	
Sulfur from food		3.44		3.44		0.82		2.46		1.64		3.28
Sulfur from $BeSO_4$				0.9						0.64		
Sulfur from urine	2.65		3.10		0.77		2.06		1.42		3.84	
Sulfur from feces	0.76	3.41	0.64	3.74	0.11	0.88	0.52	2.58	0.41	1.83	0.69	4.53
Balance		+0.03		+0.6		—0.06		—0.12		+0.45		—1.25
Daily average		+0.01		+0.08		—0.03		—0.02		+0.11		—0.16

Phosphorus Balance.

Phosphorus from food		10.40		10.40		0.44		1.32		0.88		1.76
Phosphorus from urine	1.70		1.60		0.42		1.44		1.01		1.98	
Phosphorus from feces	2.00	3.70	2.18	3.78	0.36	0.78	0.79	2.23	0.86	1.87	2.20	4.18
Balance		+6.7		+6.62		—0.34		—0.91		—0.99		—2.42
Daily average		+0.84		+0.82		—0.17		—0.15		—0.24		—0.3

TABLE IV.

Experiment I.—Data in grams.

General Summary.

Period.	Urine. Vol. cc.	Nitrogen.	Ash.	Phosphorus.	Sulfur.	Feces. Dry weight.	Nitrogen.	Ash.	Phosphorus.	Sulfur.
I. Fore period	3503	48.33	14.98	1.7	2.65	185.6	4.6	73.31	2.00	0.76
II. 1st $BeSO_4$ period	3738	46.54	15.04	1.6	3.10	147.5	3.46	87.47	2.18	0.64
III. Special period	1030	16.16	3.44	0.42	0.77	28.0	0.82	15.70	0.36	0.11
IV. Intermediate period	2748	38.65	11.30	1.44	2.06	102.5	2.75	63.93	0.79	0.52
V. 2nd $BeSO_4$ period	1957	27.59	8.64	1.01	1.42	64.0	1.50	42.24	0.86	0.41
VI. After period	3577	58.31	16.42	1.98	3.84	129.5	2.90	83.03	2.20	0.69
Daily Average.										
Fore period, 8 days.	438	6.04	1.87	0.21	0.33	23.2	0.58	9.16	0.25	0.09
1st $BeSO_4$ period, 8 days.	467	5.82	1.88	0.20	0.38	18.4	0.43	10.93	0.27	0.08
Special period, 2 days.	515	8.08	1.72	0.21	0.38	14.0	0.41	7.8	0.18	0.05
Intermediate period, 6 days.	458	6.44	1.88	0.24	0.34	17.08	0.46	10.66	0.13	0.09
2nd $BeSO_4$ period, 4 days.	489	6.89	2.16	0.25	0.35	16.0	0.38	10.56	0.22	0.10
After period, 8 days.	447	7.29	2.05	0.24	0.48	16.18	0.36	10.38	0.28	0.09

of weight accompanied by an increasing and prolonged loss of nitrogen, inorganic matter, sulfur and phosphorus as the result of the disturbance of nutrition which the beryllium sulfate induced. In this experiment the salt was diuretic in its effect.

SECOND METABOLISM EXPERIMENT.—The dog used in this experiment was the one employed in the first. Although she seemed in normal condition at the end of the last experiment a month elapsed before the second one was begun. During this time the dog appeared to be perfectly healthy and was very lively.

Diet.—The character of the diet was the same as that in the first experiment.

TABLE V.

Composition of Daily Diet. Experiment II.

Ingredients.	Lean meat.	Cracker meal.	Lard.	Infusorial earth.	Water.	Total.
Days	1–24	1–24	1–24	1–24	1–24	
	Grams.	Grams.	Grams.	Grams.	cc.	Grams.
Daily amount	178	48	35	10	415	686
Nitrogen	6.69	1.06	0.0002	0.00012		7.75
Sulfur	0.338	0.078				0.42
Phosphorus	0.16	0.06				0.22
Inorganic matter	1.96	0.62		9.7		3.45

Fore Period.—Normal conditions. Days, 1–8. Jan. 27 to Feb. 3, 1911. During this period there was a slight variation in the amount of daily urine and a small gain in weight.

Dosage Period. — Days, 9–16. Feb. 4–11, 1911. The dosage period began at 2.30 o'clock on Feb. 4. The amount of beryllium sulfate given was 0.5 gram. It was increased half a gram each day until on Feb. 6, 1.5 gram were given. On the following morning at 9 o'clock vomit was found in the cage. It consisted of a few particles of undigested meat in a mass of brownish mucus. The vomit amounted to 8 grams. This was added to the food for Feb. 7. No beryllium sulfate was given that day. The following day (Feb. 8) the dog appeared to be normal. The dose was again omitted but on Feb. 9 the dog was given 1 gram of beryllium sulfate. On the following morning the dog vomited. The vomit consisted of mucus which was added to the food. On Feb.

10 the dose was omitted; the dog was not sick. On Feb. 11, 1 gram of beryllium sulfate was given. The dog was sick the following morning but only to a slight degree—less so than at any time previous. It was decided to close the dosage period at this point as it was evident that the permissible maximum dose had been given.

After Period.—Days, 17–24. Feb. 12–19, 1911. The period consisted of eight days during which time no beryllium sulfate was given. The data for the experiment are given in Tables VI–VIII.

TABLE VI. *Experiment II.*

Fore Period.

Date. Jan.	Body weight. Kilos	$BeSO_4$. Gram.	Urine. Vol. cc.	Sp. gr.	Reactions, litmus.	Nitrogen. Grams.	Feces. Dry weight. Grams.
27	12.23		470	1016	acid	13.43	25.0
28	12.30		410	1016	acid		12.0
29	12.36		450	1016	acid	14.38	9.0
30	12.36		490	1016	acid		23.0
31	12.37		540	1015	acid	15.22	16.0
Feb. 1	12.32		490	1017	acid		13.0
2	12.34		510	1015	acid	11.71	8.0
3	12.34		485	1016	acid		24.0
			Beryllium Sulfate Period.				
4	12.33	0.5108	500	1016	acid	14.56	16.5
5	12.31	1.0078	510	1016	acid		11.5
6	12.30	1.5117	450	1020	am. ac.	14.48	25.3
7	12.28		485	1018	acid		10.5
8	12.29		430	1020	acid	15.57	19.5
9	12.33	1.0200	495	1017	acid		none
10	12.36		390	1018	acid	15.51	20.0
11	12.32	1.0114	555	1017	acid		27.3
			After Period.				
12	12.27		540	1017	acid	15.00	8.4
13	12.32		460	1018	acid		9.5
14	12.32		445	1019	acid	15.57	19.1
15	12.31		510	1017	acid		19.0
16	12.22		550	1017	am. ac.	15.68	10.0
17	12.29		350	1021	am. ac.		9.0
18	12.38		415	1017	acid	15.40	27.2
19	12.34		535	1017	acid		5.2

TABLE VII.

Experiment II.—Data in grams.

Nitrogen Balance.

	Fore period, 8 days.		$BeSO_4$ period, 8 days.		After period, 8 days.	
Nitrogen from food		60.96		60.96		61.61
Nitrogen from urine	54.74	57.47	60.48	3.65	61.65	64.64
Nitrogen from feces	2.73		3.17		3.01	
Balance		+3.49		—2.69		—3.03
Daily average		+0.44		—0.33		—0.38

Balance of Inorganic Matter.

Ash from food		98.24		98.24		98.24
Ash from urine	17.28	103.73	19.20	104.09	20.48	90.61
Ash from feces	86.45		84.89		70.13	
Balance		—5.49		—5.85		+7.63
Daily average		—0.68		—0.73		+0.95

Sulfur Balance.

Sulfur from food		3.36		3.36		3.36
Sulfur from $BeSO_4$				0.91		
Sulfur from urine	3.12	3.80	3.88	4.76	3.48	4.08
Sulfur from feces	0.68		0.88		0.60	
Balance		—0.44		—0.49		—0.72
Daily average		—0.05		—0.06		—0.09

Phosphorus Balance.

Phosphorus from food		1.76		1.76		1.76
Phosphorus from urine	1.76	3.71	1.69	3.90	1.98	3.46
Phosphorus from feces	1.95		2.21		1.48	
Balance		—1.95		—2.14		—1.7
Daily average		—0.24		—0.27		—0.2

TABLE VIII.

Experiment II.—Data in grams.

Summary of Results.

Period.	Urine. Vol. cc.	Nitrogen.	Inorganic matter. Grams.	Phosphorus.	Sulfur.	Feces. Dry weight. Grams.	Nitrogen. Grams.	Inorganic matter. Grams.	Phosphorus. Grams.	Sulfur. Gram.
I. Fore period	3845	54.74	17.28	1.76	3.12	130.0	2.73	86.45	1.95	0.68
II. $BeSO_4$ period	3815	60.48	19.20	1.69	3.88	130.6	3.17	84.89	2.21	0.88
III. After period	3805	61.65	20.48	1.98	3.48	107.4	3.01	70.13	1.48	0.60
				Daily average.						
Fore period, 8 days.	480.6	6.84	2.16	0.22	0.39	16.2	0.34	10.8	0.24	0.085
$BeSO_4$ period, 8 days.	476.8	7.56	2.40	0.21	0.48	16.3	0.40	10.6	0.28	0.110
After period, 8 days.	475.6	7.71	2.56	0.25	0.44	13.4	0.38	8.8	0.18	0.08

Discussion of Results.—The fore period showed that the animal continued to gain weight on the prescribed diet as in the former experiment. During the period in which 5 grams of beryllium sulfate were administered there was a fluctuation in weight which continued into the after period and a slight decrease in the total volume of urine. The daily volume varied considerably but these variations usually attended pronounced sickness as the result of the dosage. The dosage period showed a marked increase in the elimination of nitrogen and phosphorus, but the sulfur and total inorganic matter were not much affected.

The after period showed a continued and increased elimination of body nitrogen. The weight of the animal fluctuated during this time but by the end of the period there was a return to normal. There was an increased elimination of sulfur but less phosphorus than in the dosage period. The data for the analysis showed the same nutritive disturbances as in the first experiment although not to so marked a degree.

THIRD METABOLISM EXPERIMENT.—The animal used in this experiment was a medium sized short haired dog weighing nearly 10 kilos.

Diet.—The diet was of the same character as in the two former experiments.

TABLE IX.

Composition of Daily Diet. Experiment III.

Ingredients.	Lean meat.	Cracker meal.	Lard.	Infusorial earth.	Water.	Total.
Days	1–18	1–18	1–18	1–18	1–18	
	Grams.	Gram.	Grams.	Grams.	cc.	Grams.
Daily Amount	160.0	0.40	30.0	7.0	350	587.0
Nitrogen	6.4	0.88	0.0018			7.3
Sulfur	0.30	0.06				0.36
Phosphorus	0.16	0.05				0.21
Inorganic matter	1.6	0.32		6.79		8.71

Preparatory Period.—This period consisted of 15 days. It was two or three days before the dog became accustomed to its new environment and ate all the daily amount of food. During this time there was great fluctuation in the amount of daily urine. On the last day of the period there was complete retention of the urine and the large volume on the first day of the fore period was the extra elimination.

Fore Period.—Normal condition. Days, 1–6. May 16–21, 1911. With the exception of the fluctuation in the daily volume of urine normal conditions were maintained throughout the period.

Dosage Period. — Days, 7–12. May 22–27, 1911. On the 7th day beryllium sulfate was given *per os* just before feeding as in the previous experiments. The dosage was started with 0.5 gram and gradually increased until on the third day of the period the dog received 1 gram. This was followed by very slight vomiting. The same dose was repeated on the following day. Slight vomiting resulted the next day at noon. This was frothy and unformed. On each of the two days following the dog received 1 gram of the sulfate with the food. The vomit as a result each time was stringy and mucous-like. The decided increase in amount showed that the dog was at the point of very marked effect from the beryllium sulfate and the dosage was stopped.

After Period.—Days, 12–18. May 28 to June 2, 1911. During this period normal conditions were maintained.

Analytical Results.—The analytic data pertaining to this experiment are recorded in Tables X–XII.

TABLE X.

Experiment III.

Fore Period.

Date. May.	Body weight. Kilos.	$BeSO_4$. Gram.	Urine. Vol. cc.	Sp. gr.	Reaction, litmus.	Nitrogen. Grams	Feces. Dry weight. Grams.
16	9.60		475	1019	acid	13.05	7.0
17	9.68		250	1015	acid		none
18	9.70		320	1020	acid	12.86	12.2
19	9.73		355	1017	acid		8.4
20	9.75		355	1017	acid	14.2	10.6
21	9.63		455	1020	acid		19.2

Beryllium Sulfate Period.

22	9.65	0.5049	310	1020	acid	12.6	9.6
23	9.66	0.7543	300	1021	acid		11.4
24	9.72	1.0055	340	1017	acid	14.3	9.5
25	9.65	1.0293	440	1019	acid		11.7
26	9.65	0.9890	400	1020	acid	15.4	8.8
27	9.75	1.0000	275	1010	acid		13.4

After Period.

28	9.65		525	1021	acid	15.0	19.7
29	9.68		325	1015	acid		9.8
30	9.64		390	1020	acid	14.3	10.0
31	9.69		325	1020	acid		15.1
June							
1	9.86		220	1051	acid	14.3	none
2	9.68		490	1020	acid		20.2

TABLE XI.

Experiment III.—Data in grams.

Nitrogen Balance.

	Fore period, 6 days.		$BeSO_4$ period, 6 days.		After period, 6 days.	
Nitrogen from food		43.8		43.8		43.8
Nitrogen from urine	40.11	41.9	42.3	43.8	43.6	45.5
Nitrogen from feces	1.8		1.5		1.9	
Balance		+1.9		±		—1.7
Daily average		+0.33		±		—0.03

Balance of Inorganic Matter.

Ash from food		52.26		52.26		52.26
Ash from urine	10.6	42.89	10.32	49.6	10.56	55.77
Ash from feces	32.29		39.28		45.20	
Balance		+8.37		+2.66		—3.51
Daily average		+1.39		+0.44		—0.58

Sulfur Balance.

Sulfur from food		2.16		2.16		2.16
Sulfur from $BeSO_4$				0.7		
Sulfur from urine	2.16	2.46	3.13	3.50	2.47	2.86
Sulfur from feces	0.30		0.37		0.39	
Balance		—0.30		—0.64		—0.70
Daily average		—0.05		—0.10		—0.11

Phosphorus Balance.

Phosphorus from food		1.26		1.26		1.26
Phosphorus from urine	1.25	1.33	0.89	1.0	1.20	1.33
Phosphorus from feces	0.08		0.11		0.13	
Balance		—0.07		+0.26		—0.07
Daily average		—0.01		+0.04		—0.01

TABLE XII.

Experiment III.—Data in grams.

General Summary.

Period.	Urine.						Feces.			
	Vol. cc.	Nitrogen.	Inorganic matter.	Total phosphorus.	Total sulfur.	Dry weight.	Nitrogen.	Inorganic matter.	Total phosphorus.	Total sulfur.
I. Fore period	2210	40.11	10.6	1.25	2.16	57.4	1.8	32.29	0.08	0.30
II. Dosage period	2065	42.3	10.32	0.89	3.13	64.4	1.54	39.28	0.11	0.37
III. After period	2275	43.6	10.56	1.20	2.47	74.8	1.91	45.20	0.13	0.05
Daily Average.										
Fore period, 6 days.	368.3	6.68	1.76	0.20	0.36	9.56	0.30	5.38	0.01	0.05
Dosage period, 6 days.	344.1	7.05	1.72	0.14	0.52	10.73	0.25	6.54	0.01	0.06
After period, 6 days.	379.1	7.26	1.76	0.20	0.41	12.46	0.31	7.53	0.02	0.008

Discussion of Results.—With this animal the 4 grams of beryllium sulfate produced little variation in weight and total volume of urine for the period. The results of the analysis, however, show the same effects on nutrition as were produced in the two former experiments. The eliminations of body nitrogen, total inorganic matter, sulfur and phosphorus were affected, extending beyond the dosage period. The increased loss of nitrogen manifested itself more in the after period.

The results obtained in the third experiment corroborated those of the first and second.

General Remarks.—From the three experiments performed to ascertain the effect of beryllium sulfate on metabolism, it was clearly shown that the salt produced pronounced nutritive disturbances. It was demonstrated in the fore period of each experiment that the daily diet was more than sufficient for the animal and the disturbed metabolism which developed during the dosage and after periods must have been due to the direct influence of the beryllium sulfate.

The metabolic disturbance was shown before sufficient amounts of the salt were given to produce vomiting as was illustrated in the first dosage period of the first experiment.

III. Pharmacological Effects.[1]

Some earlier workers in endeavoring to establish the atomic weight of beryllium and its place in the periodic system made a few comparative studies of the pharmacological effect of salts of beryllium, aluminium, barium, strontium and calcium. As early as 1882, Blake asserted that the general physiological effects of beryllium were similar to those of aluminium.[2]

[1] The pharmacological experiments are in progress at this writing (May 14, 1912).

[2] Blake: "Atomic Weights of Beryllium as Determined by its Physiological Actions." *Chemical News*, lxv, p. 111. Ueber den Zusammenhang der molekularen Eigenschaften anorganischen Verbindungen und ihre Wirkung auf den lebenden thierischen Organismus. Berichte der deut. chem. Gesellschaft, xiv, p. 394.

He used the sulfate salt and found that 0.023 gram per kilo of body weight was a lethal dose. His experiments were performed on dogs but he does not describe the symptoms produced.

In 1884, Brunton and Cash[1] in studying the comparative toxicity of the alkali earths found that irritation of the spinal cord was marked in cases of poisoning by beryllium. Beryllium, calcium, strontium and barium all paralyzed the motor nerves to some extent. These workers used beryllium chloride in their experiments and performed them on frogs. They found that the substance acts as a poison with 0.0013 gram of $BeCl_2$ to 1 gram of body weight of frog. The table on the following page is a summary of their results.

In a study of the comparative toxicity of aluminium and beryllium, Siem[2] found that subcutaneous and intravenous injections of beryllium were more toxic than those of aluminium. He used solutions of the lactate and tartrate salts of beryllium and performed his experiments on frogs, cats, dogs and rabbits. He calculated his doses in terms of BeO and states that from 0.02–0.028 gram BeO was a lethal dose for a medium sized frog; from 0.004–0.005 gram BeO per kilo of body weight for dogs and cats and from 0.008–0.01 gram per kilo of body weight for rabbits. Only in one experiment with a frog in which the injection was made in the dorsal lymph-sac was the dose without effect. In all other experiments the animal died in a few days.

In the frogs he found the effect of beryllium to be as follows: A central paralysis due to the direct effect on the ganglia of the brain and spinal cord. The reflexes were impaired and the breathing became irregular. The action of the heart was rhythmical but grew gradually slower. The frogs died on the day following the injection.

In other animals the toxicity of the beryllium was accompanied by loss of appetite and impaired activity of

[1] Brunton and Cash: Royal Society, Philosophical Transactions, 1884, p. 197.

[2] Siem: "Ueber die Wirkung des Aluminiums und Berylliums auf den thierischen Organismus." *Inaug. Dissert.*, Dorpat, 1886.

Notes from Table of Relation of the Alkalies and Alkaline Earths as Poison to Nerve and Muscle.

Only Beryllium Data Given.

Substance.	Animal.	Dose or application.	Symptoms in brief.	Post mortem appearances.	Reactions.	Antagonisms.
Beryllium chloride.	Frog (16 g.) (15 g.).	Injection 0.02. Injection 0.014.	Reflex may be increased soon after injection. There is a general start if any part of body is touched. Movement may be tremulous and swaying. If left hind leg is no longer drawn up there may be a rhythmic twitch of limb muscles. Heart beating up to end. Respiration at first slight-	Heart beating feebly.	Usually complete paralysis of the nerve and very faint (if any) response of muscle to tetanizing current.	Potassium increases altitude of beryllium curve. Calcium does not increase extent of the contraction.

	ly accelerated.	
Local application 1–100, 5 min. to 20 min.		Rapidly reduces altitude of curve. Causes an increase of tonus (contracture) in muscle exhibited whilst resting not after stimulation. Usually shortens curve slightly. Reduces double summit to single. In 45 min. muscle ceases to contract.

the intestines usually after a single diarrheal bloody discharge. There was vomiting, marked physical depression and rapid loss of body weight and temperature. At first the animal became lethargic, lying on its side and showing great reluctance to move. When this was done the animal showed ataxic movements. This condition was followed by general muscular tremor of short or long duration accompanying a complete loss of sensation. The animal usually died in a convulsion. The heart beat became weak but remained rhythmical and its action outlasted the respiration always by a minute. The motor nerves and muscles remained irritable up to the time of death. The amount of urine excreted was slight and usually contained albumin.

Siem administered the beryllium either subcutaneously or intravenously. Beryllium tartrate was used for the subcutaneous injection and the double salt of beryllium lactate for intravenous injections. The animals usually died on the third day.

Pathological examination showed the liver and kidneys to be much affected. The former was a deep yellow color, the parenchyma soft and fragile, without granular structure, and bloodless. A microscopic examination showed the cells of the liver to have been destroyed giving place to a yellowish red, finely granular mass. There was also a decided congestion of the alimentary canal, especially in the case of the dogs. The entire mucous membrane of the stomach and particularly of the small intestine was deep red and swollen. The intestine showed marked ulceration. Siem found that the general effects of beryllium were the same as that of aluminium except that the toxic effects of beryllium were more pronounced.

In the investigation carried on in this laboratory it was decided to first ascertain the effects of the beryllium sulfate if a calculated toxic dose was given *per os*. The material used in all these experiments was a Kahlbaum preparation.

In the study of its effect on nutrition, it was found that 0.1 gram of beryllium sulfate per kilo of body weight, given

per os produced sickness in a dog. This was equivalent to 0.014 gram of BeO.

First Pharmacological Experiment.—Dose given *per os*. A short haired dog weighing 6.08 kilos was chosen for this experiment. The animal was lively and in good condition. Four times the amount of the toxic dose was administered. As this was of considerable bulk, it was divided and given in three balls of meat, one immediately after the other. Each one was swallowed without mastication.

July 22, 1911. 11.30 A.M. 2.38 grams of $BeSO_4$ given.
12.30 P.M. Dog lying quietly in cage but apparently well. Stood up without difficulty.

1.00 P.M. Vomited most of the meat in solid masses.

2.30 P.M. Dog apparently well.

5.30 P.M. Ate a little food and drank water.

6.00 P.M. Dog seemingly normal again.

Second Pharmacological Experiment.—Dose given *per os*. The same animal was used in this experiment as in the former one. Three days were allowed to elapse before renewing the dosage. During that time the dog appeared to be perfectly normal—eating and retaining all the food given. In this second experiment the amount of beryllium sulfate was four times that given in the former one. The dose was administered in five balls of meat.

July 25, 1911. 2.30 P.M. 8.95 grams of $BeSO_4$ given.

3.00 P.M. Vomited, apparently all of the meat. Frothy liquid in the bottom of the cage.

3.05 P.M. Vomit, white frothy liquid.

3.15 P.M. Dog quiet. Stood in cage without difficulty.

3.30 P.M. No symptoms.

4.00 P.M. No symptoms.

Conclusion. From the above results it was apparent that the beryllium sulfate caused vomiting before a sufficient amount of the material was absorbed to produce a fatal result or even strong toxic symptoms

Third Pharmacological Experiment. *Subcutaneous Injection.*—Siem states that from 0.004 to 0.005 gram of BeO per kilo of body weight is a lethal dose for a dog. He made

daily injections of small amounts until the lethal dose was administered. In the following experiments the whole dose was given in single injections. In the first experiment the amount administered was equivalent to 0.003 gram of BeO per kilo—slightly below the lethal dose as determined by Siem. This was done to ascertain what symptoms would be produced.

The animal used in the previous pharmacological work was used in this experiment. The dog was in perfectly normal condition and lively. The $BeSO_4$ was dissolved in the smallest possible amount of physiological salt solution and the injection made over the right kidney, the area being previously shaved and thoroughly cleansed.

July 29, 1911. Dog weighing 6.05 kilos. 3.45 P.M. 0.14 gram of $BeSO_4$ injected subcutaneously.

4.30 P.M. Dog lively. Fed at this time. All food taken and retained. Feces hard and dry.

Midnight. Dog gradually becoming more reluctant to rise and evidently getting sick.

July 30. 10.00 A.M. Dog lying quietly in cage, no attempt to move. When lifted to her feet the animal showed signs of pain. Breathing deeper and slower. Shivered occasionally. Nose warm.

These conditions continued through the morning but during the afternoon they began to disappear. She moved with difficulty but otherwise appeared normal. The nose was warm and albumin was found in the urine. All the food was taken and retained. During the following day the animal became more lively.

On the third day following the injection an edematous swelling developed under the groin on the side in which the injection was made, the fluid extending under the abdomen.

August 2. 3.30 P.M. 0.28 gram of $BeSO_4$ was given by subcutaneous injection over left kidney. No symptoms were produced on the day the injection was given. On the following morning, the dog was lying quietly in the cage reluctant to move, but otherwise in the same condition as before the administration of the beryllium sulfate. The breathing

was natural; the edema produced by the first injection had become slightly less. On the third day following the injection on the left side an abscess developed at the point of the second injection.

FOURTH PHARMACOLOGICAL EXPERIMENT.—A second experiment with subcutaneous injection was made repeating the conditions of the former. The dog weighed 6.8 kilos.

Aug. 5, 1911. 11.00 A.M. 0.667 gram of $BeSO_4$ was given subcutaneously over the right kidney. By the afternoon the animal seemed reluctant to stand and had difficulty in moving the right hind leg. There was slight shivering, deep breathing and considerable saliva. Food was refused. Albumin appeared in the urine voided late in the afternoon.

Three days after the administration of the beryllium sulfate decided gangrene developed on the side of the injection: the gland under the right groin became swollen. No other symptoms developed.

Aug. 11, 1911. 3 P.M. 1.285 grams $BeSO_4$ were injected over left kidney. The same results were obtained. Necrosis extending over the thigh developed on the side of injection two days after the administration of the dose.

FIFTH PHARMACOLOGICAL EXPERIMENT. *Subcutaneous Injection of Beryllium Lactate.* — Because of the marked contrast in results obtained in the above experiments and those reported by Siem it was decided at this point to change the method employed to conditions more nearly those used by him.

The lactate salt was prepared. This was done by dissolving the beryllium sulfate in the smallest possible volume of water making the solution alkaline with 1 per cent. sodium hydroxide and then faintly acid with lactic acid. This solution was used for the subcutaneous injection.

Aug. 12, 1911. Dog weighing 5.8 kilos. 12.00 M. 1.17 grams $BeSO_4$ (as lactate) were injected subcutaneously in right side. No symptoms were produced until the following day when a large swelling appeared under the right groin. The place of injection had become large and soft. No other symptoms developed. The animal appeared otherwise normal. There

was no sign of pain in the affected area. The dog's nose was cold.

Sixth Pharmacological Experiment. *Intravenous Injection.*—According to Siem[1] 0.004–0.005 gram of BeO per kilo of body weight is a lethal dose for a dog or cat. In his first experiment with an intravenous injection he administered 0.04 gram BeO to a cat weighing 2.520 kilos. The injection was made at ten o'clock in the morning. By noon of the same day the animal had become apathetic and reluctant to move. It drank some milk but this was soon vomited. On the following morning a general weakness had developed. The cat was staggering and the senses much affected. During the night there had been more vomiting. This consisted of a white, frothy mass. By noon of this day the senses were practically deadened. At his time respiration was 48, the pulse 220–230 and very weak, the temperature 34.7° (98.4 F.). On the third day the animal died.

In the second experiment Siem injected 0.1 gram of BeO into a dog weighing 16.650 kilos at three o'clock in the afternoon. During the night there was much vomiting and diarrheal feces. On the second day the dog weighed 15.630 kilos. It staggered about and dragged the hind part of the body. When the animal lay still on its side, it showed nervous twitching of the muscles. The senses were decidely dulled. The pulse was 100 and rather strong; the temperature 35.8° (96.4 F.). On the third day the weight had decreased to 14.340 kilos; the pulse was 160; the dog lay perfectly motionless, the senses entirely deadened. At 2 o'clock the respiration was very superficial and the animal died in a sudden convulsion.

In the first experiment of this research made with an intravenous injection, the amount given was a little more than the lethal dose prescribed by Seim. 30 cc. of 1 per cent. aqueous solution of beryllium sulfate was administered. The dog weighed 6.2 kilos, and the amount injected equalled 0.0067 gram BeO per kilos. The Kahlbaum preparation

[1] Loc. cit.

of the salt was used and the solution made up with distilled water. The conditions of the experiment were as follows:

April 4th, 1912. Dog weighing 6.2 kilos.

3.15 P.M. Preparation begun.
3.45 Respiration, 36.
3.46 Pulse, 132.
3.47 Injection started.
3.47–3/4 Injected 1 cc.
3.50 Injected 2 cc; respiration, 32; pulse, 132.
3.53–1/2 Injected 3.5 cc.
3.54 Respiration, 32.
3.55 Pulse, 140; injected 5 cc.
3.56 Injected 5.6 cc.; injection stopped.
3.57–1/2 Pulse, 144; irregular.
3.58 Heart more regular; respiration, 28; slowing.
3.58–1/2 Injection started again.
4.00 Injected 7 cc.
4.1 Pulse, 142.
4.2–1/2 Very rapid heart action. Injected 8 cc.; long inhalation.
4.3 Breathing very shallow.
4.3–1/2 Respiration, 28; injected 8.2 cc.; injection stopped.
4.5 Heart action rapid and weak.
4.6 Heart very irregular.
4.8 Pulse, 140; respiration, 32 (0.001 gram BeO given).
4.11 Injection started again.
4.12 Shivering tendency at the beginning has steadily increased.
4.14 Respiration, 26; irregular—shivering increasing now affecting fore legs.
4.16 Respiration, 32.
4.16–1/2 Injected 14 cc.; abdomen getting pink; salivation increasing.
4.21 Respiration, 28; injected 18 cc.
4.22 Temperature, 102.6; injected 18.6 cc.
4.34 Tremor increasing; extending over entire body.
4.35 Dog becoming uneasy.

4.38 Respiration, 52; injected 25 cc.; wagged tail when called; made effort to get off board.

4.42 Temperature, 102.6; injected 30 cc. Injection stopped; respiration, 48; heart weak and irregular; pulse, 144. Abdomen continues pink.

4.50 Put in cage, wagged tail, nose cold, apparently normal. Heart beat normal, strong; respiration, 32.

5.10 Temperature, 103.4.

5.12 Fed. All food taken.

5.34 Temperature, 102.6.

7.30 Temperature, 105.2.

When the dog was seen late in the evening it had vomited twice. The material was soft but not liquid. Since being returned to the cage 275 cc. of dark urine had been passed which contained a trace of albumin. The feces were soft. The animal lay in the corner of the cage refusing to stand. The temperature was 105°, the respiration 28 and the pulse fast. At 9 A.M. on the following day, the feces contained bloody mucous; the urine (175 cc.) was light in color and responded very strongly to the albumin test. The temperature was still 105°; the weight 5.68 kilos. The dog stood when lifted but walked about the cage with difficulty. During the afternoon the dog became decidedly depressed, refused food but drank a little water. At 9 o'clock in the evening the temperature was 104; urine 50 cc.

April 6th, 10 A.M. Temperature, 100.2. Vomit frothy and mucous. Weight, 5.51; urine 160 cc. Dog stood and walked about the cage more easily than on previous day. Refused food and water. Nose cold. At noon the dog tried to vomit, there were repeated attacks of retching and much froth about the mouth. The animal walked easily about the cage. During the day there was increased depression.

6.30 P.M. Pulse, 100; respiration, 16; cold.

8.15 P.M. Dog died in asphyxia.

An autopsy the following day showed the heart in diastole; blood very "black"; large intestine greatly congested and small intestine practically empty and flabby; stomach contained bloody, thin fluid. The bile was *thick* and black; the

lungs, liver, kidneys, pancreas, and spleen normal. The bladder was empty.

Before an experiment was tried on another dog, the new animal was kept under observation for a week and the daily volume of urine, weight of the dog and character of the feces recorded. The second experiment was performed under the same conditions but half the amount reported by Siem as the lethal dose was injected. 16.4 cc. of a 1 per cent. solution of beryllium sulfate was injected into a dog weighing 8.89 kilos which equaled 0.0025 gram BeO per kilo.

The conditions of the experiment were as follows:

April 18, 1912. 3.15 P.M. Operation begun.
3.36 Pulse, 138; respiration, 28.
3.38 Pulse, 134; respiration, 36.
3.40 Pulse, 128.
3.42–1/2 Respiration, 32; pulse, 128.
3.43 Injection begun.
3.45 Pulse, 120; somewhat irregular.
3.46 Injected 3 cc.
3.47 Pulse, 108; irregular.
3.48 Respiration, 28; injected 6 cc.; dog trembling.
4.51 Injected 11 cc. Muscular trembling increased; heart action weaker.
4.53 Respiration, 24. Tremor in muscles prevents getting pulse; abdominal surface warm and pink; nose cold.
4.55 Injected 14 cc.
4.58 Injected 17 cc. Difficulty in breathing; heart beat cannot be felt on chest.
4.59 Injected 18 cc. (by correction for amount left in tubing 16.4 cc.).
5.08 Heart very weak.
5.10 Experiment finished.
5.11 Weight 9.26 kilos; dog in cage.
5.52 Respiration, 32; pulse, 116; temperature, 102.1.
6.10 Diarrheal feces. 100 cc. urine, no albumin.
April 19. 8.45 A.M. Temperature, 105.7; pulse, 144; urine, 330 cc. dark, no albumin, feces soft but normal. Food taken and retained.

12 M. Temperature, 105; pulse, 136.

1 P.M. Temperature, 105.4; pulse, 140; urine, 50 cc. albumin.

3.15 P.M. Nose warm and dry. Dog markedly less lively.

5.30 P.M. Temperature, 105.7; pulse, 124; irregular.

7.00 P.M. Urine, 50 cc., light, no albumin, feces hard.

April 20, 8 A.M. Urine, 80 cc., slight albumin, reduction; pulse, 148; regular; temperature 104.7, the animal showed occasional muscular trembling. All food and water refused. These same conditions continued through the day except that during the afternoon the temperature dropped to 103.6.

April 21, 10 A.M. There had been bleeding from the dog's wound during the night. The animal was decidedly sick and refused all food. There was considerable frothy vomit on the pan and bloody, diarrheal feces.

4.00 P.M. Very little sign of life. Animal perfectly quiet.

4.10 P.M. Animal dead.

The autopsy the following day showed the heart in diastole and full of blood; lungs congested; blood "black"; spleen large and black along border. The large intestine was not congested, the contents were black; the small intestine was empty and flabby. The kidney, pancreas and liver were normal.

In both dogs the stomach and intestinal contents and bile were saved and analyzed for the presence of beryllium. In the first animal the element was found only in the feces and urine. It appeared in the urine voided the morning after the operation and continued to be excreted during the second day following. In the second experiment traces of beryllium were found in the contents of both the large and small intestines as well as in the urine and feces. It appeared in the urine voided the morning following the operation. The volume of urine excreted the day following the operation was largely in excess of the normal amount for that animal.

Discussion of Results.—Practically none of the symptoms described by Siem were produced in these experiments by the subcutaneous injection of the concentrated solution.

The animals showed a temporary soreness in the area of injection and stiffness of the hind leg on the treated side, but this soon disappeared. Very marked edema and necrosis set in within two or three days after the injection in all the animals used and after keeping them about ten days for observation they were chloroformed. The beryllium sulfate injected in the concentrated solutions led to a direct destructive effect upon the tissues rather than a systemic poisoning.

It was found that the Kahlbaum preparation of beryllium sulfate was far more poisonous in its effects in the experiments with *intravenous* injections than the tartrate salt used by Siem. In the second experiment, although it took longer for the general effects to be produced, they were more severe in the end. The second dog was apparently recovering from the dose the day before the final, toxic effects were the most powerful.

In both the experiments with intravenous injections the symptoms were the same. The beryllium sulfate had a very marked effect upon the heart, making the action very rapid, irregular and weak. During the operations such extreme muscular trembling occurred that before the animal was removed from the board it was impossible to count the pulse or respiration. Directly following the operation there was a decided increase of body temperature followed by a decrease about 24 hours before the death of the animal. There was a marked increase in the volume of urine. In the second experiment this extra elimination amounted to 250 cc. on the day following the operation, but was followed by a complete retention.

The feces were bloody and diarrheal, and continued so throughout the experiment. There were no evident signs of influence on the senses although no special tests were made. In neither dog were there convulsions.

IV. Effect on Enzyme Action.

1. Effect on the Activity of Sucrase.

The sucrase was precipitated from a water solution of yeast,

the precipitate washed with alcohol, dissolved in distilled water and reprecipitated. The water solution of the second precipitate was used in the experiment. A 2 per cent. solution of sucrose free from reducing substances and a 5 per cent. solution of beryllium sulfate were used. The tubes were arranged in the following order.

TABLE I.

Tubes.	I.	II.	III.	IV.	Control V.	Control VI.	Control VII.
Sugar (2%)	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.
Water	3 "	2 "	1 "	0 "	0 "	0 "	4 "
$BeSO_4$ (5%)	1 "	2 "	3 "	4 "	5 "	0 "	0 "
Enzyme solution	1 "	1 "	1 "	1 "	0 "	5 "	1 "
Per cent. $BeSO_4$	0.5%	1.0%	1.5%	2.0%	0 "	0 "	0 "
Results	Heavy reduction	Good reduction	Slight reduction	Very slight reduction	No reduction	Heavy reduction	Heavy reduction

The tubes were placed in a water bath at 38° for twenty minutes; the effect of the beryllium sulfate was tested by comparing the relative action of the contents of the different tubes on Benedict's reducing reagent. The results showed that there was a slight but marked decrease in reduction as the percentage of beryllium sulfate in the mixture increased. An amount equal to 1.5 per cent. in the solution showed a decided inhibitory effect.

2. EFFECT ON THE ACTIVITY OF PTYALIN.

To a 1 per cent. solution of starch paste a 5 per cent. solution of beryllium sulfate was added in varying proportions. 2 cc. of filtered saliva were added to each tube and the tubes allowed to stand in a water bath at 38° for half an hour. The effect of the beryllium sulfate was tested by comparing the relative power of the solution to reduce Benedict's reagent. The following table shows the proportion of the mixtures and the results:

TABLE II.

Tubes.	I.	II.	III.	IV.	Control V.	Control VI.	Control VII.
Starch	4 cc.	4 cc.	4. cc	4 cc.	4 cc.	4 cc.	4 cc.
Water	3 "	2 "	1 "	0 "	1 "	1 "	5 "
$BeSO_4$ (5%)	1 "	2 "	3 "	4 "	5 "	0 "	0 "
Saliva	2 "	2 "	2 "	2 "	0 "	5 "	1 "
Per cent. $BeSO_4$	0.5%	1.0%	1.5%	2.0%	"	0 "	0 "
Results	Slight reduction	Very slight reduction	No reduction	No reduction	No reduction	Heavy reduction	Heavy reduction

The inhibitory effect of beryllium sulfate on the action of saliva was very marked. The tubes were allowed to stand for two hours longer but practically no further action occurred. As beryllium sulfate is a strongly acid salt the results were as would be expected in this particular case.

3. EFFECT ON THE ACTIVITY OF PEPSIN.

To artificial gastric juice (0.5 per cent. pepsin in 0.2 per cent. hydrochloric acid) the 5 per cent. solution of beryllium sulfate was added in varying proportions. A shred of fibrin was added to each tube and all the tubes placed in a water bath at 38° for half an hour. The digestion was tested by the relative disappearance of the pieces of fibrin. The folfowing table gives the results:

TABLE III.

Tubes.	I.	II.	III.	IV.	Control V.	Control VI.	Control VII.
Fibrin	Shred	Shred	Shred	Shred	Shred	Shred	Shred
Water	8 cc.	7 cc.	6 cc.	5 cc.	5 cc.	5 cc.	9 cc.
$BeSO_4$ (5%)	1 "	2 "	3 "	4 "	5 "	0 "	0 "
Pepsin solution	1 "	1 "	1 "	1 "	0 "	5 "	1 "
Per cent. $BeSO_4$	0.5%	1.0%	1.5%	2.0%	0 "	0 "	0 "
Results	Slight digestion	No digestion	No digestion	No digestion	No digestion	Strong digestion	Digestion

At the end of half an hour there was slight digestion in the first tube and in the control but practically no disintegration of the fibrin in any of the other tubes. At the end of two hours the results were the same. This experiment was of decided interest as the acid reaction of the beryllium sulfate would not interfere with the action of pepsin as it would with ptyalin.

The tubes were allowed to stand for 24 hours and at that time digestion had occurred in all the tubes containing the pepsin solution and beryllium sulfate. This amount of digestion decreased with the increase percentage of beryllium sulfate. There evidently was a marked inhibitory effect upon the enzyme but not destruction.

4. Effect on the Activity of Trypsin.

Artificial pancreatic juice was made by extracting the fresh pancreas with 30 per cent. alcohol. The extract was carefully neutralized and made alkaline with 0.5 per cent. Na_2CO_3. The 5 per cent. solution of beryllium sulfate was added in varying amounts and a shred of fibrin added to each tube. All tubes were allowed to stand in a water bath at 38° for thirty minutes and the digestion determined by the relative disintegration of the fibrin.

The following table gives the conditions and results:

Table IV.

Tubes.	I.	II.	III.	IV	Control V.	Control VI.	Control VII.
Fibrin	Shred	Shred	Shred	Shred	Shred	Shred	Shred
Water	8 cc.	7 cc.	6 cc.	5 cc.	5 cc.	5 cc.	9 cc.
$BeSO_4$ (5%)	1 "	2 "	3 "	4 "	5 "	0 "	0 "
Trypsin solution	1 "	1 "	1 "	1 "	0 "	5 "	1 "
Per cent. $BeSO_4$	0.5%	1.0%	1.5%	2.0%	0 "	0 "	0 "
Results	Very slight digestion	No digestion	No digestion	No digestion	No digestion	Good digestion	Slight digestion

At the end of half an hour digestion had taken place in two of the tubes used as controls. There was very slight

digestion in the first tube containing the beryllium sulfate and no digestion in the other tubes. After allowing the tubes to stand 24 hours the results were the same.

Conclusion.—It is evident that beryllium sulfate has an inhibitory effect on the activity of all the enzymes used in the above experiments but sucrase was much less sensitive than ptyalin, pepsin or trypsin.

V. Precipitation of Proteins.

The effect of beryllium sulfate on the precipitation of proteins was made with the following solutions:

1. Egg Albumin.

The white of a fresh egg was diluted with an equal quantity of distilled water, the solution passed through cheesecloth and filtered. A 5 per cent. solution of beryllium sulfate was added a drop at a time. Controls were made with 5 per cent. solution of sodium chloride and ammonium sulfate and 1 per cent. solution of picric acid. The beryllium sulfate caused no precipitation of the albumin but compared with the sodium chloride and ammonium sulfate solutions there was a very slight turbidity.

2. Mixed Proteoses.

Mixed proteose were formed by peptic digestion of meat. The mixed proteoses were precipitated by complete saturation with ammonium sulfate and the proteoses dissolved in water. It was found that beryllium sulfate produced no precipitation of these products.

3. Gelatin.

A 2 per cent. solution of gelatin was made and 5 per cent. solution of beryllium sulfate was added, 1 cc. at a time. There was no precipitation of the protein.

4. Acid Meta-protein.

The blood and saline material were removed from hashed lean meat by allowing the meat to stand in running water over night. At the end of that time the meat was placed in 2 per cent. HCl for 24 hours. The material was then squeezed through cheesecloth and filtered. The filtrate containing the acid meta-protein was brought nearly to the

point of neutralization and the solution of beryllium sulfate added. Controls were made with sodium chloride and picric acid. The beryllium sulfate produced no effect upon the precipitation of the acid meta-protein.

Conclusion.—Proteins are not precipitated from their solutions by beryllium sulfate.

VI. Effect on Growth of Seedlings.

Sistini[1] performed experiments on the effect of beryllium on the growth of plants by replacing the magnesium sulfate in the nutrient solution with beryllium sulfate. The solutions were made as follows: 0.21 gram potassium chloride, 0.46 gram calcium nitrate, 0.1 gram iron phosphate, 0.035 gram calcium mono-phosphate and 0.20 gram of magnesium sulfate (or 0.18 gram of beryllium sulfate). The solution was made up to a liter. This gave a 0.018 per cent. solution of the beryllium.

He states in his results that beryllium in this strength may take the place of the magnesium in the growth of the plant, but is not a complete substitute for magnesium in the production of seeds. The plants developed and bloomed but the seeds were not fertile. He recovered from the ash of the plant 2 per cent. of the beryllium given when grown in the nutrient solution and traces of the metal in the ash when the plant was grown in earth to which was added the solution of beryllium sulfate.

The following series of experiments were performed to ascertain the effect of the beryllium sulfate in varying strengths on the growth of the seedlings. Controls were made with 1 per cent. solution of sodium chloride, tap water and distilled water.

First Experiment.—Lupine seeds were soaked in water for 24 hours and grown in moss for 48 hours. At the end of that time, 15 mm. was measured from the tip of the root

[1] Sistini: Ueber einige selten in Planzen vorkommende und seither noch nicht darin gefundene chemische Elemente Spiziell über Beryllium mit Rucksicht auf einige Kultivierte Planzen. *Chem. Centr.*, **1888**, p. 1622. Orig. art. Staz. Spirem Agrar, xv, p. 290–98 (19/10). Pisa lab di. chimica agraria. *Chem. Centr.*, **1888**, p. 1622.

and that distance with a dot of India ink. The seedlings were then suspended in the different solutions in such a manner that the root was immersed exactly to the distance marked off. The growth was determined by the difference in measurement from the ink mark to the tip of the root. The difference between the daily measurement and the original 15 mm. recorded the distance of growth. Measurements were taken each day. In the following experiment the dilution of the beryllium sulfate was made with tap water:

TABLE I.

Dates.	Tap water growth. mm.	Distil. water growth. mm.	NaCl 1 % growth. mm.	$BeSO_4$ 1 % growth. mm.	$BeSO_4$ 0.8 % growth. mm.	$BeSO_4$ 0.4 % growth. mm.
July 31	12.0	9.7	1.5	0.5	1.2	2.0
Aug. 15	25.5	21.5	6.0	1.0	1.2	2.0
Aug. 2	33.6	33.7	6.4	1.2	1 2	2.0
Aug. 15	11.5	9.5	2.0	1.0	2.3	2.0
Aug. 2	23.2	24.0	6.2	1.0	1.3	1.5
Aug. 3	40.6	28.0	6.2	1.0	1.3	1.5
Aug. 2	12.2	9.7	1.7	0.2	1.0	2.2
Aug. 3	23.2	14.0	6.5	1.0	1.0	2.2
Aug. 4	31.2	33.7	6.5	1.5	1.0	3.0

TABLE II.

Dates.	Tap water.	$BeSO_4$ sol. M/32.	$BeSO_4$ sol. M/64.	$BeSO_4$ sol. M/128.	$BeSO_4$ sol. M/256.	$BeSO_4$ sol. M/512.
Aug. 6	18.0	1.0	1.2	1.5	2.5	6.7
Aug. 7	42.0	1.0	1.5	2.5	4.7	16.5
Aug. 8	58.0	1.0	3.0	4.5	4.2	17.7
Aug. 7	17.0	1.2	1.2	2.2	2.0	3.7
Aug. 8	42.0	1.0	1.2	3.0	4.0	15.2
Aug. 9	53.7	1.0	1.5	3.2	4.5	18.7
Aug. 8	18.0	1.6	2.6	2.6	2.0	1.6
Aug. 9	40.0	1.0	2.6	2.6	2.0	1.6
Aug. 10	55.3	(Shriv- elled) dead	(Shriv- elled) dead	2.0	2.0	1.6
Aug. 9	17.5	1.0	1.5	2.5	2.0	2.0
Aug. 10	42.0	1.0	1.5	2.3	2.0	2.0
Aug. 11	58.0	(Shriv- elled)	(Shriv- elled)	2.3	2.0	2.0

Discussion of Results.—The beryllium sulfate had a very marked effect upon the growth of the seedlings as shown by the tap water control. In many cases there was a shrinking after the second day. In every case in which the beryllium sulfate was diluted with water the roots blackened and shrivelled after a few days. In the stronger solution this effect was produced within 24 hours.

SECOND EXPERIMENT.—The following experiments were made with the beryllium sulfate diluted with a nutrient solution[1] instead of with water. The nutrient solution was made as follows:

6.0 grams $Ca(NO_3)_2$.
1.5 grams KNO_3.
1.5 grams $MgSO_4$.
1.5 grams neutral potassium phosphate.
1.5 grams sodium chloride.
600 cc. distilled water.

TABLE III.

Date.	Control nut. sol.	$BeSO_4$ sol. M/2.	$BeSO_4$ sol. M/4.	$BeSO_4$ sol. M/8.	$BeSO_4$ sol. M/16.	$BeSO_4$ sol. M/32.
Aug. 11	11.2	0.0	1.7	2.0	1.5	2.0
Aug. 12	48.0	1.0	2.0	1.7	1.5	2.0
Aug. 13	64.0	1.0	2.0	1.7	1.5	2.0
Aug. 13	14.0	0.5	1.0	1.3	1.5	2.0
Aug. 14	53.0	1.0	1.0	1.5	1.7	2.0
Aug. 15	64.0	1.0	1.0	1.3	1.7	2.0

In the two experiments in which nutrient solution was used instead of tap water the roots remained white and crisp for several days although the beryllium sulfate entirely inhibited growth.

CONCLUSION. — From the experiments carried on in the manner described above beryllium sulfate to the strength of a M/512 solution had a marked inhibiting effect on the growth of lupin seeds.

THIRD EXPERIMENT.—Timothy seed was allowed to sprout

[1] McDougal: "Practical Text-book of Plant Physiology," 1911, p. 224.

on several pieces of wet blotting paper. When the growth had reached the height of 1/8 inch, 10 drops of the following strengths of beryllium sulfate were added each day and at the end of a week the heights of the growths on the various pieces of blotting paper compared.

TABLE IV.

Strength of $BeSO_4$.	Control tap water.	$BeSO_4$ M/256.	$BeSO_4$ M/512.	$BeSO_4$ M/1024.	$BeSO_4$ M/2048.	$BeSO_4$ M/4096.
Results	Good growth	Slight growth	Growth	Good growth	Good growth	Good growth

TABLE V.

Strength of $BeSO_4$.	Control tap water.	$BeSO_4$. Per cent.	$BeSO_4$. Per cent.	$BeSO_4$. Per cent.	$BeSO_4$. Per cent.	$BeSO_4$.
		0.5	0.25	0.125	0.06	0.03
Results	Good growth	Slight growth	Growth	Good growth	Good growth	Good growth

Discussion of Results.—Comparing the growth of the grass for the various strengths of the beryllium sulfate solutions with the tap water control the M/256 solution showed a very decided inhibitory effect and the M/512 solution a detrimental effect. Beyond this dilution the beryllium sulfate had little inhibitory effect on the growth.

It was very noticeable that when the experiment was finished and the plots of grass allowed to remain without attention, the plants that had been treated with beryllium sulfate became withered and yellow within 24 hours while the control which had received only tap water remained green and fresh for more than 48 hours.

VII. Effect on Bacterial Growth.

The bactericidal property of beryllium sulfate was tested by its effect on the growth of Staphylococcus pyogenes and B. coli communis. Tubes containing 10 cc. of gelatin medium were inoculated by adding 1 cc. of an emulsion of the bacteria. 1% solution of $BeSO_4$ was added in varying amounts to different tubes and the tubes plated. Colonies developed on all

the plates up to the one to which 10 cc. of the $BeSO_4$ had been added. As the beryllium sulfate is strongly acid in its reaction the inhibitory effect produced at this point may have been due to the acidity.

VIII. Summary of Conclusions.

The experiments with beryllium sulfate have shown very conclusively that the substance has a marked effect on biochemical processes. When administered with the food it produced decided nutritive disturbance, which manifested itself in loss of body weight, total inorganic matter, nitrogen, sulfur and phosphorus.

When large doses were administered *per os* the substance caused vomiting in the dog before a sufficient amount was absorbed to produce any other obvious toxic symptoms.

When the calculated lethal dose was administered by a single subcutaneous injection, the substance produced edema and necrosis of the tissue extending over a large area. No other decided symptoms were produced by this method.

Very gradual intravenous injections of the salt produced decided toxic effect. The action of the heart became irregular —usually rapid and very weak; the respiration also became irregular and shallow. During the period of injection it produced decided muscular trembling but this disappeared soon after the operation. As a direct effect of the injection the temperature increased, sometimes to 105°, but about 24 hours before the death of the animal the temperature began to decrease and steadily fell.

After the intravenous injection there was an increased elimination of urine followed by retention. The feces became diarrheal and bloody. Vomiting began about the time the dog refused food or water.

The beryllium sulfate had a decided inhibitory effect on the action of ptyalin, pepsin, and trypsin. It also retarded the action of sucrase but not to so great an extent. Solutions of the salt (1% or less) did not effect the precipitation of proteins from neutral or acid solutions.

Below the concentration of M/512 solution, the beryllium sulfate did not have an inhibiting action on the growth of lupine or timothy seedlings, but the more concentrated solutions of this salt prevented growth and had a destructive effect on the plant life.

Beryllium sulfate when present in proportions less than 0.5% had very little, if any, bactericidal action.

BIOGRAPHICAL.

Emily Cromwell Seaman was born in Brooklyn, New York, on March 5th, 1876. She received the degree of B.S. from Adelphi College in 1899, and the degree of M.A. from Columbia University in 1905. From 1901 to 1908 she taught in elementary schools in Brooklyn. She resigned this work to specialize in Physiological Chemistry and entered the Laboratory of Biological Chemistry of Columbia University in the Spring of 1908. In 1909 she was given the position of Tutor in Physiological Chemistry at Teachers College and in 1910 was appointed Instructor.

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