A CONTRIBUTION TO THE STUDY OF CALCIUM METABOLISM

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INTRODUCTORY

When an animal is deprived of inorganic salts in its food profound constitutional disturbances resulting in death are produced. The salts of the blood must not only be present in sufficient quantity to bring the osmotic pressure of the blood to a constant value but they must also be present in certain definite ratios. Every living cell of the body must be washed by a fluid containing salts of certain mono- and divalent metals in an unvarying ratio otherwise a disturbance in the intracellular ion-proteins (Loeb)² or the intracellular colloidal salts (Osborne)³ is produced. Bearing in mind this necessity for a constant ratio to exist between the various salts of the blood, a number of interesting points are raised when we consider what will be the probable effects of depriving an animal, completely or partially, of salts of one particular metal, say calcium. If the proper ratios are maintained in the blood then the following eventualities present themselves as likely to occur :—

1. The excretion of calcium is checked wholly or partially. During the progress of my research an article appeared by Goitein⁴ which disposes of this supposition, for he showed that, if a rabbit

1. Dated at Melbourne, October 7th, 1907.

2. *Dynamics of Living Matter*, New York, 1906.

3. *Journal of Physiology*, Vol. XXXIV, p. 84, 1906.

4. *Pflüger's Archiv.*, Vol. CXV, p. 118, 1906.

received less than 0·16 grammes of calcium per kilo. per day in its food, there was a steady loss of calcium from the body. Lehmann¹ has also shown that in starvation the calcium excreted exceeds the amount of this substance present in the drinking water taken.

2. The other salts of the blood are reduced *pari passu* by increased excretion. This, however, would entail a considerable fall in the total molecular concentration of the blood and as the living cells of the body and also the red corpuscles are extremely sensitive to osmotic changes this supposition may also be ruled out.

3. The deficiency in the food is made good by certain tissues of the body giving up a portion of their calcium to the blood and so keeping the proper inorganic balance in this fluid. That this would be the most probable contingency may be inferred from a number of facts. Förster² who was the first to make observations on the effect of insufficient calcium in the food found that the muscles lost 56 per cent. of their calcium content, whilst the bones also showed a considerable diminution. Voit³ found that on a calcium-poor diet the bones were more brittle, the skeleton showed a smaller percentage of dry weight than in the normal animal, and that the quantity of calcium in all the organs of the body was more or less diminished.

If such a reservoir exists in the body we might expect that its calcium content would show considerable variations and be influenced by the calcium in the food, *e.g.*, would not only be capable of yielding calcium to the blood but of storing the same if an excess of calcium were absorbed from the alimentary canal. To help the elucidation of these points three series of experiments were conducted. In the first series rabbits were placed on a calcium-poor diet and, after a suitable interval analyses were made of the percentage of total ash as also the percentage of calcium of the blood, in order to see if the ratio of the calcium to the total mineral matter of the blood actually remained

1. Abstract in *Maly's Jahresbericht*, Vol. XXIII, p. 497, 1894.

2. Abstract in *Maly's Jahresbericht*, Vol. III, p. 251, 1874.

3. *Zeit. für Biologie*, Vol. XVI, 1880.

the same as in normal control animals. The bones were also analysed in order to see if the calcium content had diminished relative to the other inorganic ingredients. In the second series metabolism experiments were conducted on myself; a simple but liberal diet was taken with excess not only of calcium but also of nitrogen. Analyses of urine were also made as regards most of its constituents. In the third series advantage was taken of a suitable case of rectal feeding in hospital in which a diet insufficient in protein but comparatively rich in calcium was administered. Analyses were made of the calcium and nitrogen in the enemata administered and in the faeces or wash-out from the bowel.

CHEMICAL METHODS EMPLOYED

Total nitrogen was determined by the Kjeldahl method; chlorides by the Volhard method; purin nitrogen by the Walker Hall purinometer; phosphates by titration with uranium acetate standardised against disodic phosphate; total sulphates and ethereal sulphates by the usual method. For determining urea the methods given by Folin¹ were ordinarily followed, but in the experiments with rectal feeding the hypobromite method was considered sufficiently accurate for urea. Sodium and potassium in urine were estimated by the method of Garratt²; calcium and magnesium in urine by the method given in Hoppe Seyler's handbook³. Calcium in food and faeces was determined by first ashing the material by the method of Neumann and then carrying out the quantitative estimation of calcium in the acid solution.⁴ Calcium in blood was determined by drying the latter, obtained from the carotid artery, and ashing with fusion mixture; bone was broken up, dried and also ashed with fusion mixture; in both cases the calcium was estimated in the usual way, namely, as oxide. The total ash of blood was determined by taking 10 c.c. from

1. *American Journal of Physiology*, Vol. XIII, p. 45, 1905.

2. *Journal of Physiology*, Vol. XXVII, p. 507, 1902.

3. *Der siebente Aufl.*, p. 420, Berlin.

4. *Ibid.*, p. 397.

the carotid artery of the anaesthetised animal, placing this in a weighed crucible, evaporating and burning over a low bunsen flame. The residue was then extracted with water, the ashing continued over a blow-pipe flame, and the watery extract added in the usual manner. For determining the total ash of bone, a weighed amount of dried bone was placed in a tared crucible, burnt, and ignited to constant weight over a blow-pipe flame. All reagents employed were tested for impurity but with negative results.

EXPERIMENTAL

I.—*Experiments with Rabbits*

The animals used for the following experiments were the ordinary brown wild rabbits which had been netted, and brought to the laboratory. Young, healthy animals of both sexes were used, care being taken to exclude lactating females, in order that the secretion of milk, which is rich in calcium, might not vitiate the results.

The control animals were fed on a green vegetable diet. This consisted mainly of grass, but lettuces and cabbages were used as well.

The experiment animals were fed on oatmeal, as it has been found that animals on this diet soon suffer from calcium insufficiency. At first the oatmeal was made into a thick paste with distilled water ; but it was found that this caked into a hard mass which was refused by the animals. So the oatmeal was given in the dry form, and a dish of distilled water for drinking was placed in the cage. During the first days of each experiment, a certain amount of green food was also given, until the animal became accustomed to the dry diet. During the later experiments, maize meal was added to the animal's diet of oatmeal and distilled water. To carry out the analyses the animals were anaesthetised with ether. A cannula was put into the carotid artery in the neck, and the animal was bled as long as the blood ran freely. Care was taken not to continue the bleeding so long that fluid would be withdrawn from the tissues and cause an error in the composition of the blood. The blood was collected in a measured vessel ; a certain amount was measured off and used to estimate the

ash of the blood, while the remainder was dried and its calcium-content was determined. After getting the blood in this way, the animal was killed by breaking the neck. The femora and humeri were then cut out, cleaned of muscles and tendinous attachments, pounded up and dried. Their total ash and calcium content were then estimated. An examination of the internal organs was made, but no definite microscopic changes were observed. In some of the animals, a hyperaemia of some of the joints was observed, the synovial membrane and cancellous tissue were engorged, but there was no effusion into the joint-cavity, nor any sign of adhesions in the joint. These changes affected mainly the larger joints, which were always more markedly altered than the smaller joints. These changes were noted in three of the animals fed on calcium-poor diet (one of which died) and in one animal fed on green vegetable diet. In these cases no endocarditis was observed, and the significance of the condition is not known.

In order to obtain a greater amount of calcium in blood, and so, if possible, to diminish the experimental error, in one of the series of experiments two animals were used at the same time in some of the later experiments. Ten c.c. of blood from each were ashed, and the rest of the blood was measured and mixed, dried, and the calcium-content determined.

Rabbit I.—Buck: was anaesthetised and killed on November 19th, 1906, after being brought from the country, where it had been feeding on the natural grasses.

Under ether, carotid artery in the neck cannulised and 48 c.c. of blood withdrawn; of these 8 c.c. were dried and ashed, and 40 c.c. dried and the calcium-content estimated. The femora and humeri were removed, cleaned, smashed up, dried; and the total ash and calcium-content determined.

Results: Bone, ash 1.0596 g. in 1.8545 g. dried bone, = 57.08 per cent.

Ca 0.5191 g. in 1.6047 g. dried bone, = 32.35 per cent.

Blood, ash 0.0502 g. in 8 c.c. = 0.627 g. in 100 c.c.

Ca 0.0026 g. in 40 c.c. = 0.0065 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{56.6}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{1.04}{100}$$

Rabbit II.—Doe: died on November 26th, 1906, after one week on oatmeal and distilled water diet. At the post-mortem examination there was synovial engorgement affecting the large joints (hips, knees and shoulders). The femora and humeri were smashed up and dried.

Results: Bone, ash 1.1243 g. in 1.8339 g. dried bone, = 61.31 per cent.

Ca 0.2178 g. in 0.8679 g. dried bone, = 25.09 per cent.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{41.08}{100}$$

Rabbit III.—Doe: killed on November 29th, 1906, after being kept in a hutch since November 19th, 1906, on green vegetable diet. 45 c.c. of blood obtained from carotid artery; of these 10 c.c. dried and ashed, and 35 c.c. dried and their calcium-content estimated. The femora and humeri were removed, smashed up and dried.

Results: Bone, ash 1.8572 g. in 2.8760 g. dried bone, = 64.24 per cent.

Ca 0.3131 g. in 0.8788 g. dried bone, = 35.61 per cent.

Blood, ash 0.0476 g. in 10 c.c., = 0.476 g. in 100 c.c.

Ca 0.0035 g. in 35 c.c., = 0.01 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{55.4}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{2.1}{100}$$

Rabbit IV.—Doe: killed December 7th, 1906, after being fed since November 19th, 1906, on oatmeal and distilled water. 30 c.c. of blood obtained from carotid artery; of these, 10 c.c. were dried and ashed, and 20 c.c. dried and their calcium-content estimated. The femora and humeri were smashed up and dried. Post-mortem: hyperaemia of the cancellous tissue and synovial membrane was observed; most marked in the knees and shoulders, less marked in the elbows and hips, and still less marked in the small joints. No endocarditis.

Results: Bone, ash 0.9619 g. in 2.0749 g. dried bone, = 46.36 per cent.

Ca 0.1810 g. in 0.9015 g. dried bone, = 20.09 per cent.

Blood, ash 0.0563 g. in 10 c.c. blood, = 0.6631 g. in 100 c.c.

Ca 0.0091 g. in 20 c.c. blood, = 0.04551 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{43.3}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{6.8}{100}$$

Rabbit V.—Doe: killed December 20th, 1906; fed since December 8th, 1906, on oatmeal and maize meal and distilled water. 22 c.c. of blood obtained from carotid artery; of these, 10 c.c. were ashed, and 12 c.c. were dried. The femora and humeri were smashed and dried.

Results : Bone, ash 1·1771 g. in 2·5805 g. dried bone, = 45·23 per cent.

Ca 0·3396 g. in 1·2874 g. dried bone, = 26·39 per cent.

Blood, ash 0·0525 g. in 10 c.c. blood, = 0·525 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{58}{100}$$

Rabbits VI and VII.—Killed January 29th, 1907, fed on green grass. 10 c.c. of blood from each was ashed; and 60 c.c. of mixed blood was dried and their calcium-content determined. The femora and humeri were cleaned, smashed up and dried.

Results : Bone, ash 1·5231 g. in 2·2982 g. dried bone, = 66·31 per cent.

Ca 0·3580 g. in 1·2510 g. dried bone, = 28·62 per cent.

Blood, Ca 0·0054 g. in 60 c.c. = 0·0090 g. in 100 c.c.

ash 0·1451 g. in 20 c.c. = 0·7255 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{43·1}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{1·2}{100}$$

Rabbits VIII and IX.—Killed March 27th, 1907; fed since January 30th, 1907, on oatmeal and maize meal and distilled water. 10 c.c. of blood from each ashed; 53 c.c. (26-27) of blood dried, and their calcium-content estimated. The femora and humeri were smashed and dried.

One of these animals showed hyperaemia of the synovial membrane of the larger joints.

Results : Bone, ash 1·0485 g. in 2·1306 g. dried bone, = 49·18 per cent.

Ca 0·3059 g. in 1·3966 g. dried bone, = 21·90 per cent.

Blood, Ca 0·0087 g. in 53 c.c., = 0·0164 g. in 100 c.c.

ash 0·1591 g. in 20 c.c., = 0·7955 g. in 100 c.c.

$$\text{Bone, } \frac{\text{Ca}}{\text{Total ash}} = \frac{44·5}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{2·06}{100}$$

Rabbit X.—Doe : killed March 28th, 1907, after being fed since January 30th, 1907, on oatmeal, maize meal and distilled water. 10 c.c. of blood were ashed; 30 c.c. dried and the calcium-content estimated. The femora and humeri were cleaned, smashed up and dried. Post-mortem showed engorgement of shoulders, hips and knees; no other joints affected; no endocarditis.

Results : Bone, ash 0·8196 g. in 1·6392 g. dry bone, = 50 per cent.

Ca 0·3196 g. in 1·6392 g. dry bone, = 19·49 per cent.

Blood, Ca 0·0046 g. in 30 c.c., = 0·0155 g. in 100 c.c.

ash 0·0833 g. in 10 c.c., = 0·8330 g. in 100 c.c.

$$\text{Bone, } \frac{\text{C}}{\text{Total ash}} = \frac{38·98}{100}$$

$$\text{Blood, } \frac{\text{Ca}}{\text{Total ash}} = \frac{1·86}{100}$$

TABLE I.—RABBIT EXPERIMENTS

Rabbit	Feeding					Bone Ca Bone ash	Blood Ca Blood ash
I ...	Green grass	...	...	...	...	$\frac{56.6}{100}$	$\frac{1.04}{100}$
II ...	Oatmeal	...	...	...	...	$\frac{41.08}{100}$	—
III ...	Green grass	...	...	...	...	$\frac{55.4}{100}$	$\frac{2.1}{100}$
IV ...	Oatmeal ...	...	...	...	...	$\frac{43.3}{100}$	$\frac{6.8^1}{100}$
V ...	Oatmeal and maize meal	...	...	...	...	$\frac{58}{100}$	—
VI and VII ...	Green grass	...	...	...	...	$\frac{43.1}{100}$	$\frac{1.2}{100}$
VIII and IX ...	Oatmeal and maize meal	...	...	...	...	$\frac{44.5}{100}$	$\frac{2.06}{100}$
X ...	Oatmeal and maize meal	...	...	...	...	$\frac{38.98}{100}$	$\frac{1.86}{100}$

It will be seen from the above table that the amount of calcium in the blood varied only between 1 and 2 per cent. of the total ash. On the other hand, considerable variations took place in the calcium content of the bones. Though the results are not quite uniform, it will be seen that the average calcium percentage of the bones of animals fed on oatmeal, and oatmeal with maize meal, is lower than that of the normal control animals fed on green stuff.

II.—*Metabolism in Man*

The experiment was continued for five days. All the estimations were made with undried substances.

Diet.—For lunch weighed amounts of biscuit (thin Captain) and butter were taken, and after the first day a measured quantity of milk was added. For tea and breakfast weighed amounts of oatmeal

1. See previous note.

with a small quantity of salt, were boiled for hours in a double saucepan and a measured quantity of milk was taken with the oatmeal.

The body-weight was taken daily at a regular hour, due precautions being taken to ensure the same clothes being worn : the same weighing machine was used throughout.

The exercise taken consisted of a four mile walk each day.

The work done during the day was the ordinary routine work of the Physiological laboratory.

About eight hours sleep was obtained each night.

The total urine passed in the twenty-four hours was collected, mixed and measured ; the specific gravity was taken and the amount then diluted to the nearest convenient whole number to avoid fractions in multiplication. Samples of this diluted urine were then used for analysis ; a few drops of chloroform were added to prevent decomposition.

The total faeces were collected, weighed, and a sample kept for analysis ; a few drops of formalin being added to prevent the growth of moulds.

As nearly as possible a full analysis of the urine was carried out, but owing to the lack of a suitable colorimeter and the expense of absolute alcohol, the estimation of the creatinin excreted could not be made.

It was intended at the outset to attempt to estimate the complete inorganic metabolism ; but it was found that no suitable method of reasonable accuracy could be obtained to estimate the sodium and potassium when present in small quantities mixed with large quantities of organic matter and phosphates. Therefore, the estimations of sodium and potassium in food and faeces were not made. The calcium was estimated in food, urine and faeces ; since accurate methods can be used for isolating and determining the calcium even when mixed with organic matter.

TABLE II

Date July, 1906	Weight kilos.	Lunch	Food Tea	Breakfast	Urine c.c.	Faeces gm.
23	71·8	Biscuit 100 g. Butter 20 g. —	Oatmeal 230 g. Salt 20·6 g. Milk 290 c.c.	Oatmeal 230 g. Salt 20·6 g. Milk 290 c.c.	1,200	248
24	72·3	Biscuit 121·5 g. Butter 20 g. Milk 400 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 348 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 232 c.c.	1,620	166
25	72·7	Biscuit 119 g. Butter 19·5 g. Milk 375 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 348 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 238 c.c.	1,705	115·5
26	72·8	Biscuit 123 g. Butter 18·5 g. Milk 325 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 348 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 232 c.c.	1,815	163·5
27	72·7	Biscuit 122·5 g. Butter 21 g. Milk 315 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 348 c.c.	Oatmeal 230 g. Salt 16·5 g. Milk 232 c.c.	1,975	98

TABLE III

DATE July, 1906	Food	CALCIUM Urine	Faeces	Food	NITROGEN Urine	Faeces
23	1·2640 g.	0·1083 g.	—	18·37 g.	12·14 g.	7·23 g.
24	1·7373 g.	0·1687 g.	1·4956 g.	21·08 g.	13·55 g.	2·85 g.
25	1·6912 g.	0·1651 g.	1·2532 g.	20·91 g.	13·60 g.	1·94 g.
26	1·6428 g.	0·1665 g.	1·3420 g.	20·63 g.	14·46 g.	2·95 g.
27	1·6311 g.	0·1680 g.	0·9276 g.	20·58 g.	14·18 g.	1·76 g.

From the above tables it will be seen that during the course of the experiment the body was not in nitrogenous equilibrium, but was retaining nitrogen. The weight of the body, it will be observed, had increased.

Intake ...	...	...	...	...	101·47 g. N
Output	...	...	...	...	84·66 g. N
Balance	...	...	...	...	16·81 g. N

This would represent a storing of about 109 grammes of proteid during the five days.

The calcium also shews a gain to the body.

Intake ...	...	...	...	...	...	6.7024 g. Ca
Output ...	...	...	...	...	...	5.6867 g. Ca
Balance ...	...	...	...	...	...	1.0157 g. Ca

The excretion of Ca by the urine shows a remarkable constancy of about 0.166 gramme during the last four days of the observations, while the faecal excretion shows a gradual diminution.

TABLE IV—URINE

Date	Amount and sp. gr.	Total N in grs.	Purin N in grs.	NH ₃ in c.c. $\frac{N}{10}$ NH ₃	Urea in grs.	Chlorides as Cl ₂ in grs.	P ₂ O ₅ in grs.
23	1200 1023	12.13	0.117	450	15.12	10.3	1.79
24	1620 1031	13.55	0.156	288	10.90	14.9	2.58
25	1705 1024	13.6	0.312	420	18.78	16.5	2.34
26	1815 1021	14.46	0.166	484	24.35	19.5	2.60
27	1975 1020	14.18	0.300	424	17.57	21.6	2.83

TABLE IV—Continued

Date	Total SO ₃ in grs.	Ethereal SO ₃ in grs.	Ca in grs.	Mg in grs.	Na in grs.	K in grs.
23	2.03	0.20	0.1083	0.1093	5.3181	3.351
24	2.26	0.17	0.1687	0.1216	8.7483	3.283
25	2.42	0.17	0.1651	0.1083	8.5568	3.1003
26	2.54	0.18	0.1665	0.1126	10.690	3.168
27	2.40	0.12	0.1680	0.1215	12.2848	2.912

This table shows a gradual but marked increase from day to day of the chlorides and sodium, with a less marked rise in the excretion of phosphates, and magnesium. On the other hand the calcium remained almost constant.

III.—*Metabolism in Rectal Alimentation*

This series of observations was carried out on a patient at the Melbourne Hospital, and I am indebted to Dr. Howard for permission to investigate the case.

M. G., *aet.* 26, was admitted to the Melbourne Hospital on the 28th August, 1906, suffering from the symptoms of gastric ulcer. Rectal feeding was carried out from the 26th August until the 18th September, the only thing allowed by the mouth was plain filtered water.

The food administered, by four-hourly nutrient enemata, consisted in solutions of plasmon in water, starch in 0·7 per cent. salt solution, and white of egg in water. These were used in varying amounts during the greater part of the experiment. It was found that the amount of plasmon calculated as necessary to keep up the nitrogenous equilibrium could not be got into solution even in the total amount of water used in the twenty-four hours, so that it became necessary to reduce the amount of plasmon injected. During the last three days various nutrient enemata were tried, with the object of increasing the calcium intake.

The patient's rectum and sigmoid colon were washed out each morning at 8 a.m., and the return, together with the faeces, if any, was mixed, measured, and a sample reserved for analysis. The total twenty-four hours' urine was collected, measured, and samples taken for analysis.

The weight of the patient was taken at 11 a.m. on the first two days, but removal to the scales caused such abdominal discomfort that weighing had to be discontinued.

On the 1st September, the patient commenced to menstruate, and this function continued for four days, during which the nutrients were continued but the urine and faeces were not analysed.

In the samples of urine and faeces obtained the total nitrogen and total calcium were estimated, and the urea of the urine was approximately determined by the hypobromite method.

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TABLE V—RECTAL FEEDING

DATE Aug., 1906	WEIGHT Kilos	FOOD				WATER		URINE C.c.	BOWEL RETURN		
		Plasmon	Starch	Egg-white	5 per salt sol.	By bowel	By mouth		Faeces	Washout	Total
		Grns.	Grns.	C.c.	C.c.	C.c.	C.c.			C.c.	C.c.
25	—	—	—	—	—	—	—	300	—	—	—
26	55.9	86	11.5	114	170	910	340	1070	—	824	824
27	55.5	86	11.5	85	170	1160	170	1140	—	454	454
28	—	86	11.5	71	170	1160	200	1350	350	460	810
29	—	86	11.5	57	225	1160	280	2205	—	895	895
30	—	56	11.5	57	170	1130	280	2500	—	1050	1050
31	—	56	11.5	85	200	990	250	1595	—	700	700

Sept.

1-4 On same diet.

5	—	56	11.5	58	225	1075	225	1335	—	1400	1400
6	—	56	11.5	64	170	1075	250	1330	—	1150	1150
7	—	56	11.5	57	170	1075	140	1400	—	525	525
8	—	56	11.5	57	170	1075	200	1555	—	630	630
9	—	56	11.5	57	170	1075	250	1035	225	1035	1260
10	—	56	11.5	57	170	1075	425	1770	—	575	575
11	—	56	11.5	57	170	1075	310	1650	200	1230	1430
12	—	56	11.5	57	170	1130	170	1350	130	525	655
13	—	56	11.5	57	170	1075	250	1430	—	765	765
14	—	56	11.5	57	170	1075	250	1450	—	1005	1005
15	—	56	11.5	57	170	1075	170	1180	—	835	835
16	50	Peptonised milk				850 c.c.	280	870	230	1180	1410
17	—	Pepd. mk. 570c., stch. 23g. sal.				340 c.c.	250	925	—	1220	1220
18	—	Stch. 23g., 4 eggs, saline				990 c.c.	250	1035	—	845	845

TABLE V (*continued*)—RECTAL FEEDING

DATE Aug., 1906	CALCIUM			NITROGEN			Urea gr.
	Food gr.	Urine gr.	Faeces gr.	Food gr.	Urine gr.	Faeces gr.	
25	—	0·1978	—	—	6·88	—	14·12
26	1·5530	0·2040	0·1146	12·47	14·51	0·72	—
27	1·5492	0·1186	0·0363	11·92	9·14	0·23	18·27
28	1·5473	0·2133	0·2366	11·72	12·36	2·29	22·14
29	1·5455	0·2813	0·5156	11·40	17·32	3·13	32·7
30	1·0090	0·2859	0·1841	7·80	15·57	5·73	29·9
31	1·0127	0·2474	0·0478	8·32	11·16	1·76	20·9
Sept.							
5	1·0090	—	—	7·80	—	3·52	—
6	1·0099	0·2588	0·3506	7·93	9·36	2·28	17·2
7	1·0090	0·2652	0·0309	7·80	10·15	0·42	19·5
8	1·0090	0·2629	0·1896	7·80	10·97	0·94	19·8
9	1·0090	0·2434	0·8910	7·80	8·40	4·02	15·8
10	1·0090	0·3008	0·2556	7·80	9·84	1·27	18·8
11	1·0090	0·2798	0·1638	7·80	7·60	3·20	14·3
12	1·0090	0·3086	0·1571	7·80	6·40	1·31	11·7
13	1·0090	0·2941	0·0953	7·80	6·54	0·78	11·0
14	1·0090	0·2853	0·5919	7·80	7·67	4·22	14·0
15	1·0090	0·1961	0·2925	7·80	6·14	1·77	12·0
16	0·9936	0·2313	0·2429	4·74	8·30	2·36	13·5
17	0·6663	0·2033	0·1539	3·18	5·39	1·39	10·1
18	0·3082	0·2582	0·0136	4·29	5·56	0·53	10·7

The preceding table may conveniently be divided into three periods:—

1. From 26th to 31st August inclusive, during which the patient was fed on varying amounts of plasmon, starch and egg-white. On the first days, probably not all the plasmon weighed out was actually injected, since the solution formed a thick jelly which could only with difficulty be made to run through the nutrient tube.

Intake	...	...	8·2167 g. Ca	...	63·63 g. N ₂
Output	...	...	2·4855 g. Ca	...	93·92 g. N ₂
Balance	...	...	+ 5·7312 g. Ca	...	— 30·29 g. N ₂

It is interesting to note that the urine excreted on the day the patient had no food contained 0·19 gramme Ca, while the amount

excreted during the rest of the experiment varied between 0.11 and 0.30 gramme Ca.

2. During the next period, Sept. 6th to 15th inclusive, the patient had settled down and the results are more valuable, since the nursing staff had become accustomed to the routine necessary, and further, there was now no difficulty with the plasmon solution gelatinising in the nutrient tube during administration.

Intake	...	...	10.0909 g. Ca	...	78.13 g. N ₂
Output	...	...	5.7133 g. Ca	...	103.28 g. N ₂
Balance	...	... +	4.3776 g. Ca	...	-25.15 g. N ₂

3. During the third period a varied diet gave the results :

Intake	...	...	1.9681 g. Ca	...	12.21 g. N ₂
Output	...	...	1.1032 g. Ca	...	24.53 g. N ₂
Balance	...	... +	0.8649 g. Ca	...	-12.32 g. N ₂

Over the whole period the results are :

Intake	...	...	20.2757 g. Ca	...	153.97 g. N ₂
Output	...	...	9.3020 g. Ca	...	221.73 g. N ₂
Balance	...	... +	10.9737 g. Ca	...	-67.76 g. N ₂

The nitrogen shews a steady leak from the body. This shews that it was impossible to give by the bowel sufficient, even of a highly nitrogenous substance like plasmon, to keep the body in nitrogen equilibrium.

The urinary calcium shews a remarkable constancy throughout the experiment.

DISCUSSION AND SUMMARY

In the experiments in which rabbits were fed on oatmeal and maize meal, a diet which admittedly leads to calcium starvation, the ratio of the calcium of the blood to the total ash of the blood remained much the same as that found in the normal animal. That is to say, the blood underwent no loss of calcium relative to the other salts in the time allotted to the experiment—a result which one might anticipate from the immense importance of the salt ratios of the

blood. The ratio of calcium to the total mineral matter in the bones was, however, inconstant, and shewed fairly wide fluctuations even in the normal animal. The bones can, without doubt, act as reservoirs of calcium (and possibly of magnesium). That they lose calcium when the animal is placed on a calcium-poor diet has been proved so conclusively by others, that I have not thought it necessary to carry out confirmatory experiments. My results, however, tend to show that the bones can lose calcium relatively to the other salts, that is, by a selective autolysis and not by an autolysis of bone in mass. The experiments on my own metabolism show that calcium can be readily stored during nitrogen retention. More interesting, however, are the experiments with rectal feeding, where calcium was stored despite a continuous drainage of nitrogen from the body. In this latter case, as the proteid absorbed from the food was insufficient, the muscles and glands must have been diminishing in bulk, and yet calcium was retained. This fact rather points to the bones as the seat, in this case, of calcium storage.

In the experiments on myself and in those with rectal feeding, it will be noticed that, with a fixed diet, the urinary calcium varied but slightly, and the variations such as they were ran parallel with the total amounts of urine excreted. This result is not remarkable if we assume that the kidney, in order to lighten its work against osmotic pressure, allows a fraction of each of the salts of the blood to escape into the urine. The greater the volume of the urine, therefore, the greater the mass of salts eliminated.

Results of quantitative determinations of certain urine constituents are given as metabolic data of some importance.

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