From our experiments, we found, as the above descriptions and table indicate, that each gum, when precipitated from an aqueous solution by alcohol, gives a characteristic precipitate. This characteristic precipitate is easy to recognize and, consequently, gives a valuable index and, in most cases, definite knowledge as to which gum was in solution. The above method is an easy, rapid and definite method for the differentiation of one gum from another.

FACTORS INFLUENCING CALCIUM BALANCE.*

I. Influence of Potential Alkalinity on the Utilization of Supplementary Calcium Lactate in the Mature Rat.

BY VERSA V. COLE,[1] JOHN H. SPEER AND FREDERICK W. HEYL.

It is generally accepted that the path of excretion of calcium is influenced by the intake of other salts, due to their influence either upon absorption from the gut or to their influence upon retention in the tissues.

Mendel and Givens (1), working with dogs, found that the addition of 6.5 Gm. of sodium bicarbonate per day had no significant effect upon the calcium balance. They report also that large doses of sodium bicarbonate (40 Gm. per day) did not decrease the urinary output of calcium in a diabetic man. Sato (2) studied the effect of alkali on the retention of calcium in infancy, using doses of 2 and 3 Gm. of sodium bicarbonate added to milk. He came to the conclusion that it produced a distinctly unfavorable effect upon calcium balance.

The unfavorable influence of alkali used as sodium bicarbonate on the calcium balance is perhaps due to its influence in limiting the absorption of calcium and phosphorus in the gut by raising the p_H and converting the calcium into relatively insoluble salts in the upper small intestine. It is stated by Zucker and Matzner (3) that the p_H of the intestinal contents of rachitic rats tended to be alkaline, and alkaline feces were obtained. Babbott, Johnston and Haskins (4) note the influence of gastric acidity upon the absorption of calcium. Conditions of hypoacidity are said to reduce the absorption of calcium (5).

It may well be, therefore, that the measurements heretofore reported on the influence of alkaline bicarbonates on calcium balance may be a composite result of an unfavorable influence in the gut which conceals any favorable influence which the potential alkalinity of the bicarbonate might have produced.

Indeed where experimental study on calcium retention has considered the influence of alkalinity and has secured this without alkaline bicarbonates, favorable influence on positive calcium balances have been reported. Thus Bogert and Kirkpatrick (6), studying the effect of base-forming diets upon calcium metabolism, found that, while acid-forming diets caused a marked increase in urinary calcium, base-forming diets produced a decided diminution in urinary calcium. Re-

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[1] Holder of the Upjohn Coöperative Fellowship at Kalamazoo College (1927–28). This paper is based upon the thesis presented by Miss Cole to the Faculty of Kalamazoo College, in partial fulfilment of the requirements for the degree of Master of Science.

tention of calcium was favored on a basic diet. The degree of basicity of these diets ranged from 55 to 54 cc. of normal alkali. Calculated as potassium citrate this amounts to approximately 6.0 Gm. per day. Shohl (7) found a base-forming diet essential to calcium storage in the young. Baumann and Howard (8) and Chaney and Blunt (9) found that calcium assimilation was decidedly benefited by orange juice. In the last-mentioned experiments, 700 cc. of orange juice per day were used, which is equivalent to somewhat less potassium citrate than the diet used by Bogert and Kirkpatrick.

There is little doubt that increasing the potential alkalinity of the tissues, which have suffered acidotic effects in several well-known clinical conditions, has a favorable influence upon calcium balance. The effectiveness would appear to be a matter of degree depending upon the condition of the subject. Thus in an ill-nourished, toxic condition, such as the case of pernicious vomiting of pregnancy reported by Halverson, Mohler and Bergeim (10), a very conspicuous retention due to alkalies is due to the fact that a slightly increased calcium retention is augmented by the decreased drain of calcium. Thus it is difficult to state, in cases of slight negative calcium balances, whether a favorable influence of potential alkalinity is due to their effect as a food or as a drug.

In considering the question of the influence of alkali on calcium balance, it appeared important to us to disentangle the combined effect obtained by the use of sodium bicarbonate, and to study the effect of potential alkalinity separately. This can, of course, be easily accomplished by the use of alkaline citrates which will have far less disturbing influence upon the p_H of the gut than will the bicarbonate. Thus while it has so far proven difficult definitely to establish the influence of potential alkalinity upon calcium retention by selection of alkaline food factors, we felt that this afforded a very definite approach to the problem involved.

In our metabolism experiments we worked out a calcium level close to the minimum daily requirement and studied the influence of partial and complete systemic alkalization. The results reported in Table VII and summarized at the end of this paper show conclusively that systemic alkalization favors more complete utilization of calcium and phosphorus and perhaps also of magnesium.

As the intake of calcium lactate was increased from 5 to 10 mg. per day, the utilization of phosphorus was decreased and the calcium balance was not benefited.

In the same way, on a given calcium level of 4 mg. per day, there is probably an alkali intake between the two levels of 500 mg. and 30 mg. citrate per day (which we used in these experiments) at which maximum calcium and phosphorus balances are obtained. This maximum will no doubt differ for every different level of calcium lactate intake.

EXPERIMENTAL.

Conditions of the Experiment.—The rats used, averaging in weight about 225 to 250 Gm., were maintained on the regular wheat—dried milk—sodium chloride stock diet. This was changed to the basal experimental diet one day before the animals were transferred to the metabolism cages. After a six-day fore period on the calcium deficient diet, the test period of six days followed.

The food was cooked and scattering was thus prevented. The salt increment was added in 1 Gm. of starch and always given prior to the main feeding. In

this way the interaction of the saline increment with the inorganic constituents of the basal diet was to a large extent avoided, and the rat ate the whole of the salts on an empty stomach.

The basal diet had the following composition: Casein (purified), 18; Yeast, 2; Calcium free salt mixture, 1; Starch, 79.

The calcium free salt mixture was a neutral mixture designed to furnish all the inorganic elements (except calcium) in the same proportion as found in milk ash (11). Additions of Fe, I, Mn, F and Al were made at the same level as in Osborne and Mendel's salt mixture (12). The total composition was: Na_2CO_3, 120.8; K_2CO_3, 252.7; $MgCO_3$, 41.6; HCl, 109; H_3PO_4, 186.5; H_2SO_4, 6.1; Fe citrate (15%), 12.2; KI, 0.022; $MnSO_4$, 0.087; NAF, 0.283; $K_2Al_2(SO_4)_4.24H_2O$, 0.05.

An analysis of the salt mixture gave Na, 10.6%; K, 26.67%; Mg, 2.64%; Cl, 20.3%; SO_4, 1.15%; Ca < 0.04%.

Analysis of the whole ration showed, Ca = 0.018%; P = 0.285%; Mg = 0.030; calculated acidity = 185 cc. 0.1 *N* acid per 100 Gm. of the diet.

Several preliminary balance experiments indicated that this diet, except for the calcium, just barely sufficed to keep the rats in inorganic equilibrium.

Metabolism periods were six days. Rats were kept in individual Hendryx metabolism cages. Each animal received 1 drop cod liver oil per day. Feces and urine were collected and aliquoted separately, but analyzed together. McCrudden's method for Ca and Mg was used and Fiske and Subbarrow's colorimetric method for P.

In these experiments, we first established the amount of calcium which just barely sufficed to maintain equilibrium at about 4 to 5 mg. At this level, we then studied the influence of systemic alkalinity on calcium, phosphorus and magnesium balance.

1. In the first series, after a uniform six-day period on the calcium free acidotic diet, the number was divided and to half, 10 mg. calcium as calcium lactate was fed, while to the diet of the other half there was added also 0.5 Gm. sodium citrate per day, which is calculated to alkalize the animal.

TABLE I.—DATA FOR SIX-DAY PERIODS.

	Fore period.				10 mg. Ca as lactate added			
Rat no.	Food intake.	Ca balance.	P balance.	Mg balance.	Food intake.	Ca balance.	P balance.	Mg balance.
	Gm.	*Mg.*	*Mg.*	*Mg.*	*Gm.*	*Mg.*	*Mg.*	*Mg.*
16[1]	75	−37.9	−23.3	−6.7	52	+14.9	− 2.6	−3.2
72	75	− 8.2	+ 3.7	−6.1	73	+44.8	+36.6	−1.1
92[1]	75	−52.8	−31.3	−8.7	69	+24.6	+27.2	+0.6
94	71	−62.6	+ 6.7	−0.2	30	+40.0	− 4.7	−2.5

[1] Rats receiving sodium citrate during Ca-lactate period.

At this level of calcium intake, it appears that increased potential alkalinity did not favorably influence the calcium balance. Thus comparing in Table VII the balance of No. 16 and No. 94, the rat without alkaline increment gained more calcium than the one with this addition, and the same is found true in comparing No. 72 and No. 92. In respect to phosphorus retention, alkaline increment favored

TABLE II.—DATA FOR SIX-DAY PERIODS.

Rat no.	Fore period. Food intake. Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.	4 mg. Ca as lactate added. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.
9[1]	44	−72	−84.6	−11.4	45	+ 6.4	+ 4.5	+ 0.9
16	60	−39	+ 1.0	− 1.8	45	−20.3	+ 5.3	± 0.0
47[1]	70.5	−26	+ 7.9	+ 0.6	67	+15.4	+35.8	+ 5.7
92	71.0	−35.5	−26.3	+11.0	71	+ 0.8	+ 0.4	+11.1
94	37.3	−30.0	−13.8	+ 1.6	38	+ 5.4	− 8.6	− 0.6
157[1]	63.3	−64.2	−47.7	−14.4	44	+ 9.6	+ 9.9	+ 0.9

[1] Rats receiving sodium citrate during Ca-lactate period.

it and this is also true of magnesium. We concluded that the calcium level was too high to evaluate the effect of potential alkalinity.

2. In the second series, we fed during the experimental period half the rats with 4 mg. calcium as lactate, and the others with 4 mg. calcium as lactate plus 0.5 Gm. sodium citrate per day.

Here a very decidedly favorable effect of the alklaine citrate is noted on both calcium and phosphorus retention.

3. Another series was carried out exactly as in 2, except that the calcium increment was depressed to 3 mg.

TABLE III.—DATA FOR SIX-DAY PERIODS.

Rat no.	Fore period. Food intake Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.	3 mg. Ca as lactate added. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.
9	37	−29.2	−38.8	− 5	39	− 1.4	−27.2	−3.2
16[1]	39.2	−40.1	−24.8	− 7.2	39	−17.9	−18.1	+0.4
47	54.5	−29.2	+10.7	− 6.1	55	− 7.6	− 1.5	−3.0
92[1]	51.0	−60.3	−32.0	− 6.4	50.2	−11.5	−14.2	−1.6
94[1]	30	−47.3	−59.3	....	33	+ 3.0	−21.9	−4.0
157	27.8	−41.7	−57.4	−11.6	33	− 2.8	+ 2.1	−3.5

[1] Rats receiving sodium citrate during Ca-lactate period.

These figures again reveal a favorable influence of potential alkalinity on calcium and phosphorus balance and the percentage of the intake which is utilized is no doubt increased by the alkaline increment as judged by the change in balance shown in Table VII.

4. In the preceding three experiments the daily addition of potential alkalinity amounted to 50 cc. 0.1 *N* alkali per day; the diet itself having a potential acidity of 185 cc. 0.1 *N* per 100 Gm. food, or about 18.5 cc. per day where 10 Gm. were eaten. In the fourth series given in Table IV, the potential alkalinity was increased by the addition of citrate equal to 30 mg. of sodium citrate in the test period.

Referring to Table VII, it can be seen by comparing this series with those reported in Experiment 2, that with a 5-mg. increment of calcium and much less citrate, the calcium balance is not quite as favorably influenced as with 4 mg. in-

TABLE IV.—DATA FOR SIX-DAY PERIODS.

Rat no.	Fore period. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.	5 mg. Ca.; 30 mg. citrate. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	Mg balance. Mg.
3	76	−17.4	−42	−14.3	78	+41.0	+42	+5.1
13	76	−28.0	−66	−13.8	78	+14.1	+30	+1.8
29	76	− 8.0	−65	−12.4	78	+41.2	+38	+1.0
31	76	−65.0	−92	−13.4	76	+ 5.0	+23	+2.8
47	81	−12.6	−50	−11.8	81	+43.7	+47	+2.4
157	76	− 6.2	−55	− 8.9	78	+38.5	+35	+3.2

crement, but the phosphorus balance is markedly influenced by the increased calcium intake even with less alkali.

5. In the fifth series, the work reported in 4 was repeated at a level of 4 mg. per day.

TABLE V.—DATA FOR SIX-DAY PERIODS.

Rat no.	Fore period. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	4 mg. Ca; 25 mg. citrate. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.
1	77	−51.8	−25	68	− 8.3	+ 7
2	77	−39.7	−20	75	+ 3.1	+29
3	77	−56.7	−18	75	− 8.0	+39
4	73	−32.4	− 9	58	+16.9	+28
5	77	−49.9	−45	36[1]	− 3.2[1]	− 3.9[1]
6	77	−59.7	−36	66	−10.7	+ 5

[1] Rat No. 5 was found to be sick during this period, and died a few days after its close.

This series may be compared (see Table VII) with Experiment 2, and it is seen that the increased alkalinity in the second series has somewhat favorably influenced calcium retention, though not by any means is the difference proportional to the alkaline increment. The comparatively slight addition of base in this experiment has had a markedly favorable influence on phosphorus balance.

6. For the sake of comparison, the rats used in Experiment 5 were after a time put through the same process, using during the test period an increment of 4 mg. calcium as calcium carbonate.

TABLE VI.—DATA FOR SIX-DAY PERIODS.

Rat no.	[Fore period. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.	4 mg. Ca as carbonate. Food eaten. Gm.	Ca balance. Mg.	P balance. Mg.
1	29	−24.2	−46.5	40	−9	−25
2	62	−24.5	−52	69	+1.3	− 3
3	64	−36.7	−54	70	−9.3	− 6
4	62	−21.9	−46	70	+5.6	+ 6
6	62	−26.7	−52	70	+1.3	0
7	65	−22.8	−55	70	+7.6	− 1

Comparing these balances with those found in Tables II and V, it can be concluded that at the same level of equilibrium, the calcium in $CaCO_3$ is not utilized as well as calcium lactate alone, but that it causes a far superior phosphorus absorption. The addition of alkaline bases to the lactate improves both the calcium and phosphorus assimilation so that utilization is superior to that found with carbonate.

TABLE VII.—TABULAR SUMMARY OF EFFECT OF POTENTIAL ALKALINITY ON CALCIUM, PHOSPHORUS AND MAGNESIUM BALANCES.

Rat no.	Calcium increment mg. per day.	Added alkali in cc. 0.1 *N* per day.	Added alkali as Na citrate per day.		Changes in balances due to additions of (a) calcium; (b) of calcium and Na citrate; 6-day period. Ca.	P.	Mg.
	Mg.	*Cc.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*
16	10	50	500	b	+ 53	+ 21	+ 4
72	10	..	...	a	+ 53	+ 33	+ 5
92	10	50	500	b	+ 77	+ 59	+ 9
94	10	..	...	a	+103	− 11	− 2
9	4	50	500	b	+ 78	+ 89	+12
47	4	50	500	b	+ 41	+ 28	+ 5
157	4	50	500	b	+ 73	+ 58	+15
16	4	..	...	a	+ 19	+ 4	+ 2
92	4	..	...	a	+ 36	+ 27	± 0
94	4	..	...	a	+ 35	+ 5	− 2
16	3	50	500	b	+ 22	+ 7	+ 8
92	3	50	500	b	+ 49	+ 16	+ 5
94	3	50	500	b	+ 50	+ 37	..
9	3	..	...	a	+ 28	+ 12	+ 2
47	3	..	...	a	+ 22	− 12	+ 3
157	3	..	...	a	+ 39	+ 60	+ 8
3	5	3.1	30	b	+ 58	+ 84	+19
13	5	3.1	30	b	+ 42	+ 96	+16
29	5	3.1	30	b	+ 49	+103	+13
31	5	3.1	30	b	+ 70	+115	+16
47	5	3.1	30	b	+ 56	+ 97	+14
57	5	3.1	30	b	+ 45	+ 90	+12
1	4	2.5	25	b	+ 44	+ 32	Not det.
2	4	2.5	25	b	+ 43	+ 49	
3	4	2.5	25	b	+ 49	+ 57	
4	4	2.5	25	b	+ 49	+ 37	
5	4	2.5	25	b	+ 47	+ 6	
6	4	2.5	25	b	+ 49	+ 41	
1	4	..	...	a	+ 15	+ 22	Not det.
2	4	..	...	a	+ 26	+ 49	
3	4	..	...	a	+ 27	+ 48	
4	4	..	...	a	+ 28	+ 52	
6	4	..	...	a	+ 28	+ 52	
7	4	..	...	a	+ 30	+ 54	

The results of all these experiments are tabulated in Table VII in which the total effect of the salt increment in the test period, compared with the blank fore period, is shown as the algebraic difference of the balances in these two periods. Undoubtedly there would be a certain measure of improvement in the second period were no salt at all added, due to the efforts of the organism to conserve its mineral

reserve, but the relative effectiveness of each combination is clearly brought out by this method. Such treatment of the data is necessary, since in spite of the uniform treatment of all the rats in the fore period, uniform negative balances could not be induced.

SUMMARY.

1. Adult rats fed on an acidotic, calcium deficient diet required an increment of 4 to 5 mg. calcium as lactate per day to come into calcium balance.

2. With calcium carbonate, the rat came into calcium balance at about the same level.

3. Calcium carbonate was probably entirely dissolved at this level and it gave in fact a much better utilization of the phosphorus, than was secured with calcium lactate.

4. Comparatively small additions of alkaline citrate to calcium lactate increased the percentage utilization of both calcium and phosphorus; larger additions of citrate caused still greater increase in utilization, though not in proportion to the amount of alkali fed.

5. It would, therefore, appear reasonable that calcium lactate would be better utilized when fed in conjunction with alkaline citrate.

6. Alkalinity probably had a favorable influence upon the magnesium balances.

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COLLECTION OF AND TRADE IN SENEGA ROOT, CANADA.

Senega root is grown chiefly in the Province of Manitoba, Saskatchewan and Alberta; the largest yield is in Saskatchewan. The root is gathered in the spring by the Indians who prepare it for market by washing it and then drying it in the sun. It is bought by individuals who visit the Indian camps for that purpose and these buyers in turn sell to dealers in the larger cities of the Prairie Provinces. It is estimated that over 400,000 pounds of senega root were collected during the spring of 1928; for shipment the root is tightly packed in bales of 200 pounds each.

Up to several years ago senega root from Winnipeg was shipped to Europe, especially to Germany, the shipments being made direct. However, for the past few years large quantities have been sent to New York City where it is handled by jobbers and wholesale druggists.