CALCIUM ABSORPTION IN RELATION TO GASTRIC ACIDITY (A Study of Rickets).

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Many workers have put forward the hypothesis that, since calcium salts are soluble in hydrochloric acid, increased gastric acidity will increase the calcium absorption and hence the supply to the body. The converse hypothesis has also been advanced to account for the mechanism of the failure of the calcium supply of the body in rickets. Faber⁽²⁾, in his argument in support of hydrochloric milk, puts forward this increased solubility of the calcium. The same argument has been used by Shaw and Williams⁽¹³⁾ and Gleich⁽⁵⁾ for Marriott's lactic acid milk⁽¹⁰⁾. Telfer⁽¹⁵⁾ has made experiments on dogs, showing the increase in soluble calcium salts in the stomach of dogs fed with the addition of hydrochloric acid. Jones⁽⁷⁾ has recorded three cases of rickets treated with hydrochloric acid milk, but as they were uncontrolled and two had also had other treatment, it is difficult to make any deduction from her results. Other workers (Shohl and Sato⁽¹⁴⁾) have shown that the addition of acid to the diet causes increased loss of base, especially calcium, from the body.

As the calcium absorbed from the food cannot be marked, it is impossible to say in a case of negative balance that so much of the calcium has not been absorbed and then excreted into the gut again, but, if a larger percentage is excreted through the urine, it is fair to argue that there has been an increased absorption, though finally the total excretion may be increased.

The following statements are generally accepted as correct. In active rickets, the retention of calcium is decreased and the balance may be negative (Holt⁽⁶⁾ ⁽¹¹⁾, and Glasgow school⁽³⁾). When treated by either cod-liver oil, or ultra-violet light, the retention is increased (Orr, Holt and others⁽¹¹⁾). All calcium salts that are soluble in an acid medium are available as a source of calcium for the body (Steenbock and others⁽¹⁶⁾).

Bearing these facts in mind, the following research was designed to see if it were possible to correlate a decreased acidity with a poor absorption and retention of calcium and *vice versa*. The work was divided into two parts:—

1. The effect of giving acids, or acid-forming salts, on the calcium balance.

2. The relationship, if any, between the changes in calcium retention that occur in the healing of rickets and any change in gastric acidity.

Estimations of serum calcium and the inorganic phosphates of the blood were also carried out on the same patients.

METHODS.

In estimations of gastric acidity, no definite test meals were given, but the stomach contents were removed and examined after the ordinary feed that the infant was taking. A fractional examination was made in every case. The samples removed were strained through gauze and then dialysed through collodion sacs, according to the method of Levy, Rowntree and Marriott⁽⁸⁾. The hydrogen ion concentration of the dialysate was determined colorimetrically and expressed as pH. The samples removed were also tested for free hydrochloric acid by Gunzberg's reagent and, whenever enough was obtained, titrated with N/10 soda, phenolphthalein as indicator, to give the total free and combined acidity.

For the calcium balance estimation, the child was placed on a metabolism bed after a period on a constant diet and the faeces and urine collected over periods of four days. The food, some milk preparation, was made up in bulk each day and samples estimated daily. In this way, errors arising from inaccuracies in making up the formula were avoided, as the actual mixture taken was analysed. The faeces and feeds were carefully dried, weighed, ashed and the calcium estimated by McCrudden's⁽⁹⁾ method, and the calcium in the urine estimated by Shohl and Pedley's⁽¹⁵⁾ method. The greatest care was taken to avoid loss in the collecting of the stools and urine.

At the same time, blood analyses were made for serum calcium (Tisdall's method⁽¹⁹⁾) and blood phosphates. The phosphate values were determined by Dr. Robison of the Lister Institute and it is through his kindness that we are permitted to use his figures. The blood analyses were used to confirm the X-ray diagnosis of rickets and cases giving a normal blood picture were not considered active. It is not meant to enter into any theoretical consideration of the value of these determinations, but this definition of active rickets was taken, as it was hoped that such cases would give the most helpful findings.

INVESTIGATION OF CALCIUM BALANCE IN CHILDREN RECEIVING ACID FEEDS.

This group consists of two children who were fed on hydrochloric acid milk, one child that received ammonium chloride for tetany and three children who were fed on lactic acid milk. In every case, the child was on a fixed diet for at least a week before the first period (four days) on the metabolism bed. The acid, or salt, was then added to the diet, and, after a period to allow the child to come into equilibrium with the new feed, the metabolism period was repeated. The results of the two metabolism periods and the blood findings are set out in Table I.

TABLE I.
CALCIUM BALANCE ON ACID FEEDS.

Sex and Age in months.	Body Weight in kilograms.	Feed	Weight of dried faeces grammes per day	CaO gms. per kilo of Body Weight per day			% Retention	Serum Calcium mgms. Ca per 100c.c.	Inorganic phosphates mgms. P per 100 gms. whole blood	Maximum Gastric Acidity as pH	Positive Gunzberg Free HCl.
				Intake	Output	Retention					
M 12	7.67	Milk mixture	10.4	0.173	0.125	+0.0485	+28	10.4	—	3.9	0
"	7.57	Same plus dil. HCl	21.1	0.152	0.178	-0.028	-17.4	10.5	—	2.5	+
F 24	9.1	Milk mixture	35.4	0.125	0.089	+0.036	+28.7	10.0	4.45	4.0	0
"	9.47	Same plus dil. HCl	22.0	0.123	0.154	-0.031	-25.1	10.3	3.83	2.5	+
M 12	6.0	Milk mixture	7.3	0.167	0.093	+0.075	+44.6	6.1	3.64	4.1	0
"	6.1	Same plus Am. Chloride	11.5	0.175	0.188	-0.013	-7.5	6.4	—	5.7	0
F 5½	3.6	Milk mixture	6.9	0.212	0.126	+0.086	+53.9	—	—	4.4	0
"	3.8	Same plus Lactic Acid	6.3	0.241	0.158	+0.082	+34.2	—	—	3.1	0
M 3	5.0	Milk mixture	5.6	0.156	0.106	+0.0505	+32.4	—	—	4.0	0
"	5.2	Same plus Lactic Acid	6.0	0.184	0.160	+0.024	+13.2	—	—	3.2	0
M 3	4.0	Milk mixture	10.0	0.239	0.1595	+0.080	+33.3	—	—	4.2	0
"	4.0	Same plus Lactic Acid	6.0	0.192	0.114	+0.078	+40	—	—	4.0	0

Both the children receiving hydrochloric acid milk were previously in a low positive calcium balance,—percentage retentions of 28 and 28.7 and retentions per kilo of +0.048 and +0.036 grammes calcium oxide respectively. Hydrochloric acid was added according to Faber's formula to the milk, *i.e.*, one ounce of 1/10 normal hydrochloric acid to four ounces of milk. After four days on the same to bring the child into equilibrium with the feed, the calcium balance was again determined. In both cases there was a very definite negative balance, -17.4% and -25.1% retention, or -0.026 and -0.031 grammes calcium oxide per kilo. In the first case, the daily output of dried stool was increased, but the converse took place in the other child. The gastric acidity rose, maxima, before HCl, pH 3.9 and 4.0 respectively and after HCl, pH 2.5 in both cases. Free hydrochloric acid was present in both in the second period.

As regards the blood changes, in the first case the serum calcium rose from 10.4 to 10.5 milligrammes per 100 c.c., a rise within the limits of error of the method, and in the second case from 10 to 10.3. The inorganic phosphates in the second case fell from 4.45 to 3.85 mgms. per 100 gms. of blood.

In brief, both cases showed a markedly negative balance, but the serum calcium was not lowered in either, but, on the contrary, slightly raised (all normal values). The inorganic phosphates were lowered. The question of greater excretion through the urine was not determined as there was a loss in the male case and as the second was a female, the urine was frequently mixed with the stool. On the occasions when it was collected separately, there was only a very small proportion of the total calcium excreted in the urine. A large part of the increased calcium excretion must therefore be through the gut.

The case on ammonium chloride (30 grains for the first day and 15 grains for three days and while on the bed) showed a similar negative balance (*see* Table I.). The retention changed from +44.6% to -7.5%, or in grammes from +0.075 grammes calcium oxide per kilo to -0.013 grammes per kilo. This case was complicated by constipation in the first period, the total dry faecal weight per day being respectively 7.3 and 11.5 in the two periods. The gastric acidity did not increase, but rather decreased, due apparently to increased secretion of mucus. The serum calcium again rose slightly from 6.1 to 6.4 mgms. The phosphates were not determined.

The three cases treated by Marriott's lactic acid milk, *i.e.*, 60 minims B.P. lactic acid added drop by drop to 1 pint of cold milk, did not show anything like such a marked change in their calcium balance (*see* Table I.). The first two cases were satisfactory, as the weight of faeces per day was very constant in the periods. The actual intake of calcium in both cases increased during the lactic acid periods and yet in both cases there was a definite drop in the retention values,—no negative balance, but a smaller retention, from 54% to 34% and from 32% to 13%, *i.e.*, from +0.0864 to +0.0821 and from

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+0.0505 to +0.0242 grammes calcium oxide per kilo respectively. In the third case, in the second metabolism period, the child was seedy and off its food and the percentage retention was greater, owing to the much smaller intake, though the absolute retention in grammes was smaller. The gastric acidity was increased in every case, but in none to such an extent as with the hydrochloric acid milk, and no case showed free hydrochloric acid.

The cases on acid feeds therefore all showed a negative calcium balance, or a less positive one, though in every case the gastric acidity, and thus presumably the amount of calcium in solution, was increased.

CASES OF RICKETS.

Very few cases of rickets were seen during the period of study, in fact, only eight definite cases (Table II.), all of whom were on milk feeds. It

TABLE II.

Number of Cases.	Condition.	Age in Months.	Range of maximum Acidity as pH.	Average maximum Acidity as pH.	Range of Titratable Acidity CCN/10 Acid	Presence of Free HCl Gunzberg Number of Cases.
8	Active Rickets	8—24	5.4—3.6	4.5	26—40%	0
6	Healing Rickets	8—21	4.8—2.6	3.4	40—90%	2

is seen that the average acidity increases when healing (X-ray) is taking place, the maximum acidity before healing being pH 4.5 and during healing pH 3.4.

Seven of these eight cases were studied from the point of view of their calcium metabolism and four were followed through from the active stage to a stage of healing. The diet of each was constant throughout the entire period of observation. The chemical findings are set forth in Table III. The cases will first be considered separately.

CASE I. D. G., female, aged 20 months.—A severe case with typical X-ray findings. Two metabolism periods showed that the calcium balance was negative, —0.003 grammes calcium oxide per kilo. The inorganic phosphates of the blood had the extreme values of 2.6 mgms. and 1.9 mgms. per 100 gms. at the end of the two periods respectively, and the serum calcium of 8.0 and 7.1 mgms. per 100 c.c. The gastric acidity had a maximum of pH 3.6 and 3.8 for the two periods. After treatment with cod-liver oil till marked signs of healing (X-ray) were present, a third period on the metabolism bed showed she had now an extremely good retention, +0.120 grammes calcium oxide per kilo, and the retention percentage was very high, +60.7%. The calcium excreted through the urine had changed from a minute trace to about 0.5% of the total output. The inorganic phosphates of the blood had risen to 5.31 and the serum calcium to 10.1. The gastric acidity had risen from pH 3.6 to a maximum now of pH 2.0 with free hydrochloric acid. The child had changed from a miserable limp object to a bouncing irrepressible creature.

TABLE III.
CALCIUM BALANCE AND GASTRIC ACIDITY IN CASES OF RICKETS.

Sex and Age in months.	Body Weight in kilograms.	Feed.	Weight of dried faeces in grammes per day.	CaO gms. per kilo of Body Weight per day.		% Retention.	Serum Calcium mgms. Ca per 100 cc Serum.	Inorganic Phosphates mgms. P per 100 gms. of whole blood.	Maximum Gastric Acidity as pH.	Free HCl Gunzberg.
				Intake.	Output.					
1. F. 20. Active Rickets 28.1.25 On Patent Extract of Cod-liver Oil 25.2.25 On Cod-liver Oil—healing 23.3.25	5.68 5.85 6.3	Cow's milk Rusks Orange juice	14.8 17.6 16.4	0.189 0.193 0.198	0.1915 0.197 0.077	-0.003 -0.003 +0.121	8.0 7.1 10.1	2.6 1.9 5.31	3.6 3.8 2.0	0 0 +
2. M. 12. Tetany Active Rickets 1.4.25 On Cod-liver Oil—healing 6.5.25	6.0 6.36	Cow's milk Orange juice	7.3 13.1	0.1675 0.172	0.093 0.083	+0.075 +0.089	6.1 { 10.0 11.0 }	3.64 —	4.1 3.1	0 0
3. F. 9. Active Rickets Latent Tetany 13.4.25 Spontaneous healing ... 11.5.25	7.7 8.4	Cow's milk Egg Benger's Orange juice	16.2 11.4	0.192 0.160	0.177 0.091	+0.015 +0.069	{ 6.2 6.4 } { 8.8 10.6 }	— —	4.8 3.8	0 0
4. F. 12. Active Rickets 16.3.25 On Cod-liver Oil—healing 20.4.25	6.7 6.8	Cow's milk Egg Broth Orange juice	11.5 8.0	0.082 0.155	0.052 0.067	+0.030 +0.088	{ 9.6 8.6 } 9.0	3.27 2.42 —	5.4 4.8 (once only)	0 0

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CASE II. Male, aged one year.—This case did not show a negative calcium balance when admitted with rickets (X-ray) and tetany, and the retention per kilo (+0.075 grammes calcium oxide) was not low. This metabolism period was not very satisfactory, however, owing to constipation, but could not be repeated as it was essential to treat the child. The maximum gastric acidity was pH 4.1. The inorganic phosphates of the blood were 3.64 mgms. per 100 gms., but the serum calcium was only 6.1. The case was first treated with ammonium chloride as described above, and then with cod-liver oil, and did well, but not as remarkably as the previous case. When healing was marked (X-ray), a further balance period gave a slightly increased calcium retention, which was now good (+0.089 grammes calcium oxide per kilo). The gastric acidity rose to pH 3.1, but free hydrochloric acid was never detected.

CASE III. Female, aged nine months.—A case of moderately severe X-ray rickets with latent tetany. The balance was not negative on admission, but very low (+0.015 grammes calcium oxide per kilo). The serum calcium was 6.3 mgms. per 100 c.c. The maximum gastric acidity was low, pH 4.8. Slow spontaneous healing took place (light in ward) and a further balance period showed a calcium retention of +0.069 grammes calcium oxide per kilo. The serum calcium rose to 8.8 and then 10.6 mgms. per 100 c.c. and the maximum gastric acidity to pH 3.8. The clinical condition improved considerably, but was better later when treatment was added.

CASE IV. Female, aged one year.—This case was of interest as it gave a marked X-ray picture and clinically the child was very ill; anæmia, fits (not tetany), and extremely poor muscle tone. The balance period showed a low positive balance +0.030 grammes calcium oxide per kilo. The inorganic phosphates of the blood were 3.27 at the beginning and 2.42 mgms. per 100 gms. just after the first metabolism period, and the serum calcium was 9.7 and 8.6 mgms. per 100 c.c. respectively for the same two periods; yet a few days later X-ray healing was clear, and a week later there was a marked improvement in the X-ray picture. The patient was treated with cod-liver oil and healed (X-ray) very well and rapidly. A metabolism period then showed a calcium retention of +0.088 gms. calcium oxide per kilo, and the serum calcium rose to 9.0 again. The gastric acidity findings were remarkable; during the first period the maximum was pH 5.4, during the second period one reading of pH 4.8 was obtained, otherwise the pH values were never below 5.6. Test meals were repeated time after time, but high acidity values could not be obtained. It is to be noted here that, although excellent X-ray healing was taking place, the child was still miserably ill, anæmic, having fits and very flabby.

DISCUSSION OF RESULTS.

The cases on hydrochloric acid, or acid-forming salts, appear quite sharp cut. The addition of a strong acid, such as hydrochloric, to the diet, even in the quantities used by Faber⁽²⁾ and other clinicians, produces a marked lowering of the calcium retention,—in fact, in our cases, a negative balance,—and the same remarks apply to ammonium chloride.

Various authors, engaged in the study of acid base balance, have reported findings that are pertinent to the question under consideration. Shohl and Sato⁽¹⁴⁾ gave 250 c.c. of N/10 acid a day to two infants 9 months old (our cases had 170.4 c.c.) and found an increased calcium output both in stools and urine, though the balance did not become negative (they gave 1.031 and 0.775 grammes calcium oxide respectively compared to our 1.148 and 1.166 grammes calcium oxide). Sawyer, Baumann and Stevens⁽¹²⁾ produced an acidosis by giving fats and got a marked calcium loss as a result. Givens and Mendel⁽⁹⁾ gave hydrochloric acid and thereby increased urinary excretion of calcium. Stewart and Haldane⁽¹⁷⁾ have shown that, by taking either ammonium chloride or calcium chloride, the excretion of calcium by

the urine is increased and also that the normal serum calcium value is slightly raised, a fact they attribute to the concentration of serum colloids in acidosis. Our results confirm the above mentioned authors.

Further, the investigation with lactic acid milk also indicates that such an acid, though probably entirely destroyed in the body, upsets the acid base equilibrium and leads to a slight loss of base (cf. Blatherwick and Long⁽¹⁾). In view of these findings, it is difficult to understand how the addition of hydrochloric or lactic acid to the diet of a rachitic infant can help the process of healing should this be dependent on, or run parallel with, an increased calcium retention.

Telfer⁽¹⁸⁾, working on calcium metabolism in rickets, showed that, in dogs, a greater proportion of the calcium salts of the food were in solution at a stated time after feeding when hydrochloric acid was given with the food, and therefore assumed that the absorption of calcium would be better when gastric acidity was high. This is probably correct, but the acidosis produced by giving hydrochloric acid accounts for the great loss of calcium and other bases (*see* Shohl and Sato⁽¹⁴⁾), found in infants fed on hydrochloric acid milk.

The good results obtained clinically on hydrochloric acid milk (Faber⁽²⁾ and others) probably depend on other actions, such as the control of diarrhoea with good soap stool formation, and the fact that in some cases the intake of calcium probably is sufficient and the dose of hydrochloric acid possibly low enough to prevent an actual negative balance. But, in view of our findings, it would seem unwise to give hydrochloric acid milk over long periods to either rachitic or normal children.

The fact that increasing the acidity of the stomach by adding acids does not lead to an increased retention, but the converse, does not disprove the theory that the poor retention in rickets is due to the low gastric acidity (Zucker and others⁽²⁰⁾), as the disturbing factor of adding a foreign acid may neutralise the beneficial effect of the increased absorption. If by other means the gastric acidity could be raised and shown to be associated with an increased calcium retention, this would be suggestive evidence in favour of the hypothesis. The known action of cod-liver oil, or light, in causing healing and an increased retention of calcium in cases of rickets led us to use such cases in looking for any relationship between changing calcium retention and changing gastric acidity.

The four cases studied must be examined in detail. The first case looked at alone would apparently prove the hypothesis, healing and an enormously increased calcium retention being associated with a marked change in gastric acidity, free hydrochloric acid being present in the healing state. The serum calcium was also increased. The second and third cases also support the theory, but the fourth case must be considered separately. The calcium retention was low, +0.030 grammes calcium oxide per kilo, and the gastric acidity extremely low, maximum reading pH 5.4. The X-ray plates showed remarkably good and rapid healing and the retention rose

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to +0.088 grammes calcium oxide per kilo. In this second period, except for one curve (shown on chart), the gastric acidity curves were lower than in the preceding untreated period. The maximum acidity, pH 4.8, was the same as the maximum reading in Case 3 (untreated), when the retention was only +0.015 grammes calcium oxide per kilo. Chart I. gives the curves of these cases before and after treatment. In the face of such curves,

it is difficult to believe that the increased retention is directly dependent on the increase in gastric acidity and, therefore, on the solubility of the salts, more especially when it is noted that Case 4 healed (X-ray) most rapidly, in spite of the very low level of gastric acidity.

A consideration of the gastric findings in other cases shows that infants suffering from an infection or serious illness (except persistent vomiting) have a low gastric acidity, but the majority of such cases do not develop rickets, though the infection, or illness, may be of long standing, and should therefore lead to a poor calcium supply, did this depend on a low gastric acidity.

In a previous paper a relationship between body tone and gastric acidity in infants has been suggested; this is particularly well illustrated in the rachitic Cases 1 and 4 in the present series. Case 1 came into hospital unable to stand and very flabby, she went out "bouncing" or, more technically, with extremely good activity. Case 4 came in and went out a miserable flabby child, though healed of her bony rickets. In fact, in infants as in adults, it is probably correct to say that good tone goes with good acidity and *vice versa*. X-ray examination would confirm this.

Hence, though the cases of rickets show an increased gastric acidity when healing, we would associate this rather with general increase in tone than in any way with the healing process. Doubtless an increased acidity

does increase the solubility of the salts of the diet, but this is not the limiting factor in the supply of calcium to the body, or its retention, as the body can and does obtain an adequate supply of calcium on extremely low levels of gastric acidity.

SUMMARY.

1. Calcium retention was decreased, the balance was negative, in infants fed on hydrochloric acid milk, or treated with ammonium chloride: in the first case, the gastric acidity was greatly increased and the converse occurred with ammonium chloride.

2. Lactic acid milk feeding led to a slightly increased loss of calcium. The gastric acidity was raised.

3. In healing rickets, the retention of calcium was increased, but the increase in gastric acidity which occurred in the majority of cases did not run parallel with this increased retention, but rather with the general condition of the child.

4. Gastric acidity does not appear to be a limiting factor in the supply of calcium to the body.

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REFERENCES.

1. Blatherwick, N. R., and Long, M. L., *J. Biol. Chem.*, Baltimore, 1922, LIII, 103.
2. Faber, H. K., *Am. J. Dis. Ch.*, Chicago, 1923, XXVI, 401.
3. Findlay, L., Paton, N., Sharpe, J. S., *Q. J. M.*, Oxford, 1920-21, XIV, 352.
4. Givens, M. H., and Mendel, L. B., *J. Biol. Chem.*, Baltimore, 1917, XXXI, 441.
5. Gleich, M., *Arch. Ped.*, New York, 1924, XLI, 548.
6. Holt, L. E., Courtney, A. M., and Fales, H. I., *Am. J. Dis. Ch.*, Chicago, 1920, XIX, 97 and 201.
7. Jones, M., *J. Am. Med. Assoc.*, Chicago, 1924, LXXXII, 439.
8. Levy, R. L., Rowntree, G. L., and Marriott, W. McK., *Arch. Int. Med.*, Chicago, 1915, XVI, 389.
9. McCrudden, F. H., *J. Biol. Chem.*, Baltimore, 1909-10, VII, 83 and 201.
10. Marriott, W. McK., and Davidson, L. T., *Am. J. Dis. Ch.*, Chicago, 1923, XXVI, 542.
11. Orr, W. J., Holt, L. E., Wilkins, L., and Boone, F. H., *Am. J. Dis. Ch.*, Chicago, 1923, XXVI, 362.
12. Sawyer, M., Baumann, L., and Stevens, F., *J. Biol. Chem.*, Baltimore, 1918, XXXIII, 107.
13. Shaw, H. L. K., and Williams, F. J., *Trans. Am. Ped. Soc.*, Chicago, 1922, XXXIV, 46.
14. Shohl, A. T., and Sato, A., *J. Biol. Chem.*, Baltimore, 1923, LVIII, 257.
15. Shohl, A. T., and Pedley, F. G., *J. Biol. Chem.*, Baltimore, 1922, L, 537.
16. Steenbock, H., Hart, E. B., Sell, M. T., and Jones, J. H., *J. Biol. Chem.*, Baltimore, 1923, LVI, 375.
17. Stewart, C. P., and Haldane, J. B. S., *Bioch. Journ.*, Cambridge, 1924, XVIII, 855.
18. Telfer, S. V., *Q. J. M.*, Oxford, 1924, XVII, 245.
19. Tisdall, F. F., and Kramer, B., *J. Biol. Chem.*, Baltimore, 1921, XLVII, 477.
J. Biol. Chem., Baltimore, 1925, LVI, 439.
20. Zucker, T. F., Johnson, W. C., and Barnett, M., *Proc. Soc. Exp. Biol. and Med.*, New York, 1922-23, XX, 20.