Importance of the stomach in maintaining calcium homoeostasis in the rat

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Abstract

The stomach helps to maintain calcium homoeostasis by making dietary calcium accessible for uptake in the intestines, although the effect of the stomach on calcium homoeostasis is poorly understood. We examined the effect on blood calcium of gastric surgery in the rat. Within three weeks gastrectomy and fundectomy (excision of the acid producing part of the stomach) induced a slight lowering of the blood calcium concentration. When parathyroidectomy was combined with either gastrectomy or fundectomy the blood calcium concentrations promptly dropped to values lower than after parathyroidectomy alone. The mortality was close to 100% during the first three weeks after combined parathyroidectomy and gastric surgery. It was nil in rats subjected to parathyroidectomy alone. Gastrectomised rats absorbed Ca^{2+} better than unoperated control rats, possibly reflecting the fact that the serum 1,25-dihydroxy-vitamin D concentration was raised. Gastrectomised rats had a food intake that was about 70% of that in intact rats, and the amount of dietary calcium absorbed (net absorption per kg body weight) by the gastrectomised rats was approximately 65% of that in intact control rats. We conclude that the acid producing part of the stomach is important for calcium homoeostasis, since its removal induced lethal hypocalcaemia in parathyroidectomised rats. One possible explanation for the hypocalcaemia induced by gastrectomy is a progressive calcium deficit. In addition, the loss of calcitrophic hormones originating in the stomach may contribute.

The stomach is important for the absorption of calcium. Pepsin and acid are thought to act in conjunction to generate soluble calcium from insoluble phosphate complexes in food.¹ Extensive gastric surgery is known to cause bone disorders (osteomalacia/osteoporosis), possibly

related to an impaired capacity for the utilisation of dietary calcium.²⁻⁵

In the present study we examined blood calcium concentrations after bilateral truncal vagotomy, which is known to reduce both basal and stimulated acid secretion in the rat by about 90%,⁶ antrectomy, which reduces basal and stimulated acid secretion in the rat by about 50%,⁷ fundectomy (excision of the acid producing part of the stomach), or total gastrectomy. Gastric surgery as above was performed before or after parathyroidectomy. We also studied the absorption of orally administered calcium after gastrectomy.

Methods

ANIMALS

Male Sprague-Dawley rats, weighing about 200 g at the start of the experiments, were used. They were fed standard rat food pellets (ALAB, Sweden) and tap water unless otherwise stated. The calcium concentration of the food was 12 mg/g and of the drinking water 62 mg/l.

OPERATIONS

Surgery was carried out under diethyl ether anaesthesia. Sham operation consisted of a midline abdominal incision and manipulation of the viscera. Parathyroidectomy was achieved by removal of both parathyroid glands by a pair of scissors under a magnifying lens, leaving the rest of the thyroid intact. Bilateral truncal vagotomy was achieved by cutting both vagal trunks immediately below the diaphragm and dissecting the oesophageal wall for additional fibres. A pyloroplasty was made at the same time to prevent food retention.⁸ Antrectomy was performed by removing the distal half of the glandular part of the stomach, including the duodenal bulb, and joining the stomach with the duodenum end-to-end.⁹ Total gastrectomy included removal of the whole stomach followed by oesophagoduodenostomy end-to-end. Fundectomy consisted of removing the acid producing part of the gastric mucosa and joining the rumen and the antrum.¹⁰ The mortality associated with gastrectomy was 3% and with fundectomy 10%. During the first week after these two operations there was no gain in body weight. Subsequently the curves showing body weight gain over time for gastrectomised or fundectomised rats were parallel to that for intact rats. The mean (SEM) daily intake of food during the three weeks after operation was 24 (1) g (n=6) in the sham operated rats, 17 (1) g (n=6) in the gastrectomised rats, and 17 (1) g (n=6) in the fundectomised rats. In one series of experiments

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Accepted for publication
14 December 1990

TABLE 1 The effect of gastric surgery, vagotomy, or parathyroidectomy on the concentration of blood calcium

Operation	No of animals	Ca^{2+} (mmol/l)	p	Ca_{tot} (mmol/l)	p*
Sham operation	14	1.33 (0.01)	—	2.24 (0.02)	—
Vagotomy	6	1.32 (0.01)	NS	2.24 (0.04)	NS
Antrectomy	6	1.31 (0.01)	NS	2.24 (0.04)	NS
Gastrectomy	8	1.29 (0.01)	<0.01	2.10 (0.03)	<0.001
Fundectomy	8	1.28 (0.01)	<0.01	2.05 (0.04)	<0.001
Parathyroidectomy	28	0.96 (0.01)	<0.001	1.63 (0.04)	<0.001

Note: Values are expressed as mean (SEM). Blood calcium concentrations were measured in each rat on five occasions over six weeks postoperatively. The mean value for each rat was calculated from the determinations during the six week period. Group means (shown in the table) were calculated from the individual means.

*Value for the difference between sham operated rats and rats subjected to gastric surgery or parathyroidectomy. NS=non significant.

gastrectomised rats and sham operated rats were maintained in individual metabolic cages for three to four days two to three weeks after the operation. This allowed studies of calcium intake and calcium excretion in urine and faeces.

BLOOD SAMPLING

Unless otherwise stated 50 or 150 μ l samples of blood were drawn from the tip of the tail and collected in heparinised capillary tubes. The tail was anaesthetised with lignocaine (Astra, Sweden).

CALCIUM DETERMINATION

Ca^{2+} was determined by a selective electrode (ICA 1, Radiometer, Copenhagen, Denmark) in 150 μ l samples of fresh whole blood. Total calcium was determined spectrophotometrically in 25 μ l of serum using a commercially available kit based on the conjugation of calcium with o-cresolphthalein (Calcium C, Wako Chemicals, Neuss, Germany). Ionised and total calcium were determined before each operation and one and three days and one, three, and six weeks after operation.

INTESTINAL ABSORPTION OF IONISED CALCIUM

Rats were fasted overnight before the start of the experiment. $^{45}\text{CaCl}_2$ (specific radioactivity 4–50 Ci/g, NEN-Chemicals, Du Pont, Dreich, Germany) was given orally via a gastric tube. In

one experiment $^{45}\text{CaCl}_2$ in a dose of 5 $\mu\text{Ci/kg}$ body weight (10^7 cpm/kg) was given in 1 ml saline and in another 50 $\mu\text{Ci/kg}$ (10^8 cpm/kg) was added to a suspension of 200 mg food in 2 ml of water. 50 μ l of blood was drawn 30 minutes and one, two, and three hours after administration of $^{45}\text{CaCl}_2$. The blood was added to an aliquot of solubiliser (150 μ l Soluene-350/isopropanol, 1:2). The mixture was incubated for one hour at 40°C and decolorised by adding 200 μ l of 30% hydrogen peroxide.¹¹ After two hours at room temperature, 10 ml of Picofluor-40 (Packard) cocktail was added. The vials were capped, vortex mixed, and left standing overnight at room temperature before determination of radioactivity (LKB β -counter Wallac 1214 Rackbeta).

DETERMINATION OF 25-HYDROXYVITAMIN D₃ AND 1,25-DIHYDROXYVITAMIN D

The serum 25-(OH)-vitamin D₃ concentration was determined by a high performance liquid chromatography (HPLC) method.¹² The method involves passage through Sep-Pak C₁₈ cartridges (Waters, Milford, MA, USA) followed by HPLC in two steps using first a reversed phase column (Nova-Pak C₁₈, 4 μ m 8 $\times$ 100 mm, Waters) and then a straight phase column (Nova-Pak, 4 μ m 5 $\times$ 100 mm, Waters). The samples were eluted with methanol/H₂O 70:30 (vol/vol) and hexane/isopropanol 96:4 (vol/vol), respectively. The method is specific for 25-OH-D₃,¹² 1,25(OH)₂ vitamin D₂ and 1,25(OH)₂ vitamin D₃ in serum were measured by a commercially available

Figure 1: Blood Ca^{2+} concentrations after sham operation, antrectomy, vagotomy, gastrectomy, or fundectomy. Each group comprised seven rats. Six weeks later they all had a parathyroidectomy. After three weeks the mortality was 100% in the gastrectomy and fundectomy groups and nil in the others.

Figure 2: Blood Ca^{2+} concentrations after parathyroidectomy in 28 rats. Six weeks later 14 of the rats were sham operated and the others had either a gastrectomy or a fundectomy. After three weeks the mortality was 100% in the gastrectomy and fundectomy groups and nil in the sham operated groups.

radioreceptor assay method (Incstar, Stillwater, MN, USA) after passage through a Sep-Pak C_{18} cartridge.¹³

URINARY AND FAECAL EXCRETION OF CALCIUM

Gastrectomised and sham operated rats were maintained in metabolic cages for three to four days. The amount of food and water ingested was measured. The urine and faeces from each rat was collected for each 24 hour period.

The volumes and wet weights, respectively, were determined. Faeces were homogenised (Polytron) in 1 mol/l HCl (1 g faeces/10 ml acid) and left standing for three hours at 4°C followed by centrifugation at 15 000×g for one hour at 4°C. The clear supernatant was diluted 1:50 with redistilled water. The calcium concentration in urine and faecal extracts was measured by the o-cresolphthalein method and the daily calcium output calculated. With respect to faeces, the mean value for each rat was calculated from the daily determinations during the four day period. Group means were calculated from the individual means.

Figure 3: Uptake of $^{45}\text{Ca}^{2+}$ into blood after oral administration to fasted rats. In (A) the rats received $^{45}\text{CaCl}_2$ orally in 1 ml saline; in (B) the rats received 10 times as much $^{45}\text{CaCl}_2$ in a suspension of food in 2 ml water. The rats had a sham operation, a gastrectomy or a parathyroidectomy (PTX), or were subjected to combined gastrectomy and parathyroidectomy. $^{45}\text{CaCl}_2$ was administered one week after operation and the radioactivity in the blood was followed up.

STATISTICAL ANALYSIS

Student's *t* test for unpaired values was used. $p < 0.05$ was considered significant. Only groups of four or more rats were included.

Results

Fundectomy and gastrectomy lowered blood calcium in the rat, while sham operation, vagotomy, or antrectomy were without effect. After parathyroidectomy there was the expected drop in blood calcium (Table I). Determination of ionised calcium and of total blood calcium showed much the same changes. Parathyroidectomy promptly lowered blood calcium also when performed after gastric surgery. In fact, the drop evoked by parathyroidectomy was more pronounced in the gastrectomised and fundectomised rats than in the sham operated, antrectomised, or vagotomised rats (Fig 1). After parathyroidectomy the mortality in the latter three groups was nil, while it was 100% in the gastrectomised or fundectomised rats. Total blood calcium changed in parallel with the changes in ionised calcium (data not shown).

In another series of experiments the order of the operations was reversed so that parathyroidectomy was followed by gastrectomy or

TABLE II Concentrations of forms of vitamin D in the serum

	25-hydroxyvitamin D ₃ (µg/l)	1,25-hydroxyvitamin D (ng/l)
Sham operation	33 (3) (27)	97 (2) (8)
Gastrectomy	16 (1)* (13)	136 (5)* (8)

Mean (SEM). Number of rats in parenthesis. Gastrectomised rats were killed three weeks after the operation.
* $p < 0.001$.

fundectomy. In these rats the gastric surgery induced a dramatic drop in the blood calcium concentration, which was already low because of the parathyroidectomy (Fig 2). Also, these animals died during the subsequent postoperative period, while those that had a parathyroidectomy and were sham operated survived. The changes in total blood calcium followed those in ionised calcium (data not shown).

In a third series of experiments rats were subjected to gastrectomy or sham operation. A week later they were fasted for 48 hours and given $^{45}\text{CaCl}_2$ by the oral route. Half of the rats received $^{45}\text{CaCl}_2$ in saline, the other half received it in a food suspension. The uptake of radioactivity in the circulation was monitored. The results, which are shown in Fig 3, suggested an enhanced capacity of the intestines to absorb Ca^{2+} after gastrectomy. Parathyroidectomy reduced the absorption of Ca^{2+} both in gastrectomised and intact rat. Determination of 25-hydroxyvitamin D₃ and 1,25-hydroxyvitamin D in the serum of gastrectomised and sham operated rats showed a reduction in the 25-hydroxyvitamin D₃ concentration and a rise in the 1,25-dihydroxyvitamin D concentration three weeks after gastrectomy (Table II).

Finally, the daily intake and urinary and faecal excretion of calcium in five gastrectomised and five sham operated rats in metabolic cages were followed over three to four days two to three weeks after surgery. The gastrectomised rats weighed 258 (14) g and the sham operated rats 320 (8) g. The daily intake of water was approximately 20 ml/day in the two groups. The daily food intake was lower in the gastrectomised rats than in the sham operated rats: 12 (2) g *v* 18 (1) g. Thus the mean daily intake of calcium was 145 mg (144 mg in food and 1 mg in water) gastrectomised rats and 217 mg (216 mg in food and 1 mg in water) in sham operated rats. Expressed per kg body weight, the intake was 562 mg/kg in the gastrectomised rats and 678 mg/kg in the sham operated rats. The daily faecal calcium output was lower in the gastrectomised rats than in the sham operated rats: 77 (8) mg *v* 90 (7) mg. Thus the amount of calcium absorbed in the gastrectomised rats was calculated to 68 mg (47% of the ingested calcium) while the corresponding value for the controls

was 127 mg (59% of the ingested calcium). Expressed per kg body weight, the amount of calcium absorbed was 264 mg/kg in the gastrectomised rats and 397 mg/kg in the sham operated rats. Thus the gastrectomised rats absorbed 65% of the amount of calcium absorbed by the sham operated rats. The urine volumes of the gastrectomised rats were lower than in the sham operated rats and the daily urinary calcium output was reduced to about a fifth (Table III).

Discussion

Neither vagotomy nor antrectomy affected the blood calcium concentration while gastrectomy and fundectomy induced a slight reduction. The combination of gastrectomy or fundectomy with parathyroidectomy resulted in very low blood calcium concentrations, lower than those seen after parathyroidectomy alone. Unlike parathyroidectomised rats, these rats did not survive more than three weeks, possibly because of the hypocalcaemia. The results indicate that the stomach is important for maintaining calcium homeostasis. A calcium deficit after gastrectomy may reflect an impaired capacity to convert insoluble dietary calcium into soluble calcium salts that can be absorbed in the small intestine. This suggests an important role for the acid secretory capacity of the stomach. Indeed, the amount of calcium (per kg body weight) absorbed by the gastrectomised rats was only about 65% of that absorbed by the sham operated controls. The question is whether a 35% reduction in the amount of calcium absorbed can explain the effects of gastrectomy on calcium homeostasis. We obtained no evidence that gastrectomy impairs the capacity of the upper small intestine to absorb Ca^{2+} . In fact, gastrectomised rats were found to absorb Ca^{2+} better than intact rats, which may be due to the high serum concentration of 1,25-dihydroxyvitamin D (see also ¹⁴). Parathyroidectomy reduced the capacity to absorb Ca^{2+} . This probably reflects an impairment of the vitamin D regulated calcium absorption, as the parathyroid hormone stimulates the formation of bioactive dihydroxylated vitamin D.¹⁵ Analogous to this, the enhanced capacity to absorb Ca^{2+} after gastrectomy may reflect an activated vitamin D dependent absorption.

Interestingly, gastrectomised rats excreted much less calcium in the urine and had smaller urine volumes than sham operated rats. During the observation period the water intake did not differ between the two groups. We have no explanation for what seems to be a calcium sparing effect of gastrectomy. It may merely reflect the reduced blood calcium.

Gastrectomy in humans is associated with hypocalcaemia and reduced bone mineral content.^{2,3,15-17} In fact, however, Ca^{2+} seems to be absorbed more effectively in gastrectomised patients than in normal subjects.¹⁸ The serum vitamin D concentrations are raised in such patients,^{4,18} which may explain the consequent enhancement of the ability of the small intestine to absorb calcium.¹⁸ Achlorhydria is thought to be associated with impaired utilisation of dietary calcium in humans.^{19,20} The results of the present

TABLE III Daily urinary calcium output in gastrectomised and sham operated rats

Operation	Day 1		Day 2		Day 3	
	Urine volume (ml)	Calcium output (mg)	Urine volume (ml)	Calcium output (mg)	Urine volume (ml)	Calcium output (mg)
Gastrectomy (13)	5.0 (1.0)	0.28 (0.02)	7.0 (3.0)	0.30 (0.03)	6.0 (1.0)	0.27 (0.03)
Sham operation (16)	11.0 (2.0)	1.44 (0.18)	13.0 (2.0)	1.49 (0.24)	12.0 (2.0)	1.67 (0.17)

Mean (SEM). Number of rats in parenthesis.
The rats were placed in metabolic cages three weeks after surgery.

study provide some support for this view but the role of gastric acid in calcium absorption is still far from clear.²¹

The stomach may play an important part in calcium homeostasis apart from that of producing acid to promote the utilisation of dietary calcium. Recently it was suggested that the acid producing mucosa harbours a calciotropic agent, gastrocalcin, which is released in response to gastrin and food intake.²²⁻²⁴ Gastrocalcin seems to stimulate the uptake of calcium into bone and to cause hypocalcaemia as a consequence. The effects of gastrocalcin deficiency and its contribution to the effects of gastrectomy remain to be identified. At present there is no explanation for the apparent paradox that the stomach is thought to produce a hypocalcaemic hormone while gastrectomy causes hypocalcaemia.

This study was supported by grants from the Swedish MRC (04X-1007), the Tercentenary Foundation of the Swedish National Bank, the Stohnes Foundation, and AB Hässle.

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