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Ants as Models for Assessing the Effects on Health of a $CaCO_3$ + $MgCO_3$ Mixture Used to Decrease Gastric Hyperacidity

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Abstract

Having previously found that aluminum hydroxide, used to decrease stomach acidity, has several adverse effects, we here examined the effects of a mixture of calcium and magnesium carbonates, a medicine also used as an antacid, taking again ants as models. Aluminum is not present in the living organisms while calcium and magnesium carbonates are. Unfortunately and unexpectedly, these carbonates induced many adverse effects. They decreased the ants' food consumption, general activity, orientation ability, trail following, audacity, tactile (pain) perception, cognition, escaping ability, conditioning, short and middle-term memory. However, they did not affect the ants' social relationship. The ants never adapted themselves to these adverse effects which even increased over the consumption of these carbonates. The ants developed no dependence on their consumption of carbonates, the effect of which slowly and linearly decreased over time after weaning, and vanished after 10 to 14 hours. Consequently, aluminum hydroxide nor the mixture calcium and magnesium carbonates are safe drugs. They should be used only in case of severe stomach pain, and during a short time period. For moderate pain and/or long time periods, natural alternatives should be researched for treating patients suffering from excess of stomach acidity.

Keywords: *Audacity; Food Consumption; Locomotion; Memory; Pain Perception*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per cm; °C: Centigrade Degrees; H: Height; l: Liter; mm/s: Millimeter Per Second; χ2= Chi Square; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Micro Liter; R: Radius; s: Second; min: Minute; h: Hour; t: Time; %: Percentage

Introduction

For decreasing the acidity of the stomach and the digestive track in general, drugs containing aluminum hydroxide as an active ingredient are commonly used, these medicines being among others Maalox®, Gaviscon®, Contracid® and Xolaam®. Though being efficient in decreasing the stomach acidity, they have several adverse effects [1-4]. Working on ants as models, we showed that aluminum hydroxide decreased their meat consumption, general activity, linear speed, orientation ability, trail following, audacity, tactile perception, cognition, escaping ability and conditioning capability, thus their memory. It increased their sinuosity of movement and impacted their locomotion and movement coordination. Moreover, ants did not adapt themselves to the adverse effects of aluminum hydroxide [5]. An alternative drug should thus be researched for obtaining the same beneficial effect. Such a kind of medicine effectively exists, its active compounds being calcium and magnesium carbonates, and is available under the labels Rennie®, Ofloxacin® and Magnesie Plus®.

Although information given in the directions for use joined to the packages of medicines containing calcium and magnesium carbonates do not relate severe adverse effects, unwanted effects are sometimes mentioned [6,7]. It is advised to consume these drugs for only a short time and not to consume them if you are aged. Occurrence of tiredness, digestive problems, headache, muscular weakness, confusion and nervous breakdown are also mentioned. This incited us to examine the effects of calcium and magnesium carbonates in the same manner we previously examined those of aluminum hydroxide.

We here below explain why we used ants as models, which species we used and what we know on it, and which physiological and ethological traits we aimed to examine.

Why ants can be used as models

Physiological functions as well as ethological traits are similar for most animals and for humans [8,9]. They are thus generally examined on animals used as models (fruit flies, cockroaches, bees, mice, monkeys for instance) before being studied on humans [10]. The use of insects is due to their rapid development and easy maintenance in a laboratory [11]. Hymenoptera are often used [12]. Ants too can advantageously be used [13]. Indeed, ant colonies present social regulation and labor division; their workers exchange information thanks to tactile and chemical signals (pheromones) produced by different glands [14-16]. They build sophisticated nests, take care of their brood and chemically mark the different places of their environment [16]. This is in favor of using them as biological models, among others for examining the impact of products used by humans [17].

Which species we used

We have made several physiological and ethological works on the ants of the genus *Myrmica*, studying among others their ecology, eyes morphology, angle of vision, visual perception, recruitment strategy, navigation system, learning [18], as well as the ontogenesis of some of their abilities [19]. The study of the impact of EMF on their conditioning, memory and responses to their pheromones showed that these ants could be used as models [20,21]. This was obvious when examining the impact of several products used by humans [5,22-25]. Each time, we could point out the effects observed in humans and give precision on them, and we revealed other effects from which humans may suffer. In the present work, the ant *M. sabuleti* Meinert 1861 was again used as models for examining the possible impact of a mixture of calcium and magnesium carbonates.

Which traits we examined

We examined a total of 22 physiological and/or ethological traits, 18 ones firstly on ants living under normal diet then on these ants consuming calcium + magnesium carbonates, and 4 other traits only on ants having consumed or consuming these carbonates. The 18 traits were: the ants' meat and sugar food consumption, general activity, speed of locomotion, sinuosity of movement, orientation ability, trail following, audacity, tactile (pain) perception, larvae caring, aggressiveness towards nestmates and aliens, cognition, escaping ability, visual and olfactory conditioning, visual and olfactory memory. The 4 other traits were: the ants' adaptation to the adverse effects of the two carbonates, habituation to their beneficial effects, dependence on their consumption, and the loss of the effects after the carbonates consumption was stopped.

Adaptation to a product occurs when its adverse effects decrease over its consumption. Habituation to a product develops when its sought-after effect decreases over its consumption. Dependence on a product appears when an individual consuming it prefers a diet including this product to a diet free of it.

Material and Methods

The material and methods we used were the same as those used previously. Therefore, to avoid plagiarism and long text, we here only briefly relate them, and advise readers to look for details in previous works, such as [23-25].

Let us recall that the experiments were firstly made on ants living under normal diet, then on the same ants consuming a mixture of $CaCO_3$ and $MgCO_3$, except those concerning the adaptation, habituation, dependence and decrease of the effects of the $CaCO_3$ + $MgCO_3$ mixture, which were of course made only on ants consuming or having consumed the mixture.

Collection and maintenance of ants

The experiments were made on two colonies of *M. sabuleti* collected in June 2016, in the Aise Valley (Ardennes, Belgium) and on a third colony, collected at a few meters of distance from the two previous ones, on the same site, which provided alien ants and allowed making some control experiments. The colonies were maintained in glass tubes half filled with water, and these nest tubes were set in a tray (34 cm x 23 cm x 4 cm) as usual [23,24]. The ants were provided with pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) three times per week, and permanently with sugar water provided in cotton plugged tubes. The ambient temperature, humidity, lighting and electromagnetism were optimum for the species [24,25]. The ants of the same colony are here often named 'nestmates'.

Solution of $CaCO_3$ + $MgCO_3$ given to the ants

Tablets of Rennie® containing 680 mg calcium carbonate and 80 mg magnesium carbonate were obtained from the drugstore Wera (Bruxelles). In the instructions for use, it is advised to consume 1 - 2 tablets 2 - 3 times per day. This corresponds to 2 - 6 tablets per day. We here studied the potential adverse effects of 4 tablets per day, a moderate dose. Meanly, humans drink one liter of water per day. When consuming Rennie® tablets, they thus meanly consume 4 tablets together with one liter of water. Insects and thus ants, due to their physiology, drink about 10 less water than mammals. Consequently, to be under a diet identical to that of humans consuming 4 tablets of Rennie® per day, the ants should be provided with a solution of 4 tablets in 100 ml of water, or 2 tablets into 50 ml. Two tablets of Rennie® were thus dissolved into 50 ml of sugar water (the solution usually given to the ants). The water used had a pH of 7.75 [personal measurement]. The obtained solution was kept at -25°C. When defrosted, 5 ml of that solution were poured into the kind of tube used to provide ants with sugar, and such a tube was deposited on the foraging area of each two experimented colonies. It was checked each day if the ants drunk the sugared solution of the carbonates, and they did. The cotton plugs shutting the tubes were refreshed every 2 - 3 days, and the content of the tubes was renewed every 7 days.

Meat and sugar water consumption, general activity

The ants present on the sugar water, on the *T. molitor* larvae, as well as in activity at any place of their habitat were counted six times per day during six days, at the same times o'clock each day (Table 1, Daily counts) (as in [5] among others). The mean of these daily counts was established (Table 1, Daily means), and the six daily means corresponding to ants consuming the carbonates solution were compared to the six daily means corresponding to ants under normal diet using the non-parametric test of Wilcoxon [26]. The averages of the daily means were also established (Table 1, last line).

Linear and angular speed, orientation to an alarm signal

The experiments were conducted on ants walking in their foraging area, their linear and angular speeds being assessed without stimulation, and their orientation being assessed by stimulating them with a nestmate tied to a piece of white paper (Figure 1A). The attached nestmate emitted its attractive mandibular glands pheromone. As in previous works [23-25], for the ants' speeds and for their orientation, the trajectory of 40 workers was recorded on a transparent sheet and analyzed thanks to appropriate software [27]. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it. The angular speed (in ang. deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory. The orientation (in ang. deg.) towards a given location is the sum of the successive angles made by the direction of the trajectory and that towards the location, divided by the number of measured angles. When the obtained value of orientation is lower than 90°, the animal has a tendency to orient itself towards the location; when it is larger than 90°, the animal has a tendency to avoid the location. The median and quartiles of each distribution of 40 values were calculated (Table 2, lines 1, 2, 3). The distributions corresponding to ants consuming the carbonates were compared to those corresponding to ants living under normal diet, using the non-parametric χ^2 test [26].

Trail following

The species' trail pheromone is produced by the workers' poison gland. As in previous works (for instance in [23-25]), 10 of these glands were isolated into 500 µl of hexane which was then kept for 15 minutes at -25°C. Then, 50 µl of this hexane solution was deposited, using a normograph pen, on a circumference (R = 5 cm) pencil drawn on white paper and divided into arcs of 10 angular degrees. The circular trail was set on the ants' foraging area and the ants' behavior when reaching the trail was quantified by the number of arcs of 10 angular degrees 20 ants of each colony walked along the trail (Figure 1B). The distribution of the 40 numbers obtained for the two colonies was characterized by its median and quartiles (Table 2, line 4). The values corresponding to ants consuming the carbonates solution were compared to those corresponding to ants living under normal diet using the non-parametric χ^2 test.

Audacity

As in previous works [23-25], a tower standing on a platform, both made of strong white paper (Steinbach®, the tower height = 4 cm; the tower diameter = 1.5 cm), was presented to the ants, on their foraging area, and those moving on the apparatus were counted 10 times over 10 min (Figure 1C). The mean and the extremes of the recorded values were established (Table 2). The values corresponding to the two colonies as well as those obtained in the course of two successive minutes were added (as in [5]), and the five sums obtained for ants consuming the carbonates solution were compared to the five sums previously obtained for ants living under normal diet using the non-parametric Wilcoxon test [26].

Tactile (pain) perception

The locomotion of ants on a rough substrate allows assessing their tactile perception: if they perceive the rough character of the substrate, they walk slowly and sinuously on it; if they weakly perceive such a character, they walk more quickly and less sinuously. Such locomotion was thus assessed after the ants had consumed the carbonates during four days. As previously [23-25], a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was tied to the borders and the bottom of a tray (15 cm x 7 cm x 4.5 cm). The tray was so divided into a zone 3 cm long, a following one 3 cm long containing the emery paper, and a last zone 9 cm long. Such an apparatus was prepared for each colony. To make the experiment, 12 ants of each colony were transferred into the first zone of their apparatus. When leaving that zone, they walked for a time on the emery paper. Their linear and angular speeds were then assessed as usually (see above 'Linear and angular speed') (n = 24; Table 2, line 6). The values corresponding to ants consuming the two carbonates were compared to the values corresponding to ants under normal diet using the non-parametric χ^2 test.

Brood caring

A few larvae or nymphs of each colony were removed from their nest and set in front of its entrance. For each colony, five of these larvae were observed, as well as the ants' behavior towards them (Figure 1F, 1G). The larvae among these five observed ones which were still not replaced in the nest were counted after 5 s, 2, 4, 6, 8, and 10 minutes and the numbers obtained for each colony were added (Table 3, line 1). The numbers corresponding to ants consuming the carbonates solution were compared to the numbers corresponding to ants under normal diet using the non-parametric Wilcoxon test [26].

Aggressiveness against nestmates and aliens

These traits were assessed in the course of five dyadic encounters with either a nestmate or an alien ant, as in previous works [23-25]. Each encountering occurred in a cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which were covered with talc. Each time, an ant of colony A or colony B was observed during 5 minutes and its behavior towards a nestmate or an alien ant was assessed by the number of times it did nothing (level 0 of aggressiveness), contacted the opponent with its antennae (level 1), opened its mandibles (level 2), gripped the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 1H, 1I). The numbers obtained for the two colonies were added (Table 3, lines 2 and 3), and the results corresponding to ants consuming the carbonates solution were compared to those corresponding to ants living under normal diet using the non-parametric χ^2 test. The ants' aggressive behavior was also assessed by the variable "a" = number of aggressiveness levels 2 + 3 + 4 / number of levels 0 + 1, as it has been done in previous studies [5].

Cognition

This trait was examined using a protocol set up while studying the effects of nicotine [22]. Two pieces of white paper (Steinbach®, 12 cm x 4.5 cm) were duly folded and inserted in a tray (15 cm x 7 cm x 4.5 cm) so that the tray presented a first small loggia, then a path with twists and turns, and finally a large loggia into which a piece of wet cotton had been set. Such an apparatus was built for each colony. To make the experiment, 15 ants of each colony were transferred in the first loggia of their apparatus, and after that, those present in this loggia and in the large one were counted after 30s, 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were added (Table 3, line 4). The results obtained for ants consuming the carbonates solution were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test.

Escaping ability

This trait was assessed as already done in previous studies [5,25]. For each colony, 6 ants were set under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in the ants' tray. A small notch (3 mm height, 2 mm broad) had been made in the rim of the glass bottom for giving to the ants the opportunity of escaping (Figure 1J). To assess the ants' escaping ability, those escaped and those still enclosed were counted after 30s, 2, 4, 6, 8, 10 and 12 minutes. The results obtained for the two colonies were added (Table 3, line 5), and the sums corresponding to ants consuming the carbonates were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test. As in previous works [5], the ants' ability in escaping was also quantified by the variable "n° of ants escaped after 12 min/12".

Visual and olfactory conditioning and memory

After the ants had consumed calcium and magnesium carbonates during 8 days, we used again a protocol many times employed (among others in [23-25]) for examining if these carbonates impacted the ants' conditioning and memorizing capabilities We took as control values those obtained on another similar colony during a previous study [28]. A green hollow cube was set above the entrance of the sugar water tube, and the ants began so to be visually conditioned to that green cube. After this experiment, a following one consisted in depositing pieces of basilica in front of the entrance of the sugar water tube, the ants beginning so to be olfactory conditioned to basilica. Tests were made over time, first while ants were expected to acquire conditioning, then, after removal of the cue, while they were expected to lose it. Ten ants of colony A and of colony B were individually tested after different time periods, in a Y-apparatus provided with a green hollow cube or pieces of basilica in one of its branch (Figure 1K). The Y-apparatus was made of strong white paper and was deposited in a tray (30 cm x 15 cm x 4 cm). The cue set in this Y-apparatus was randomly located in the right or in the left branch of the apparatus. Moving into the branch containing the cue was considered as giving the correct response. For each test, the response of the 20 used ants was recorded, and the proportion of correct responses calculated (Table 4). These proportions obtained for ants under one and the other kinds of diet were compared using the non-parametric Wilcoxon test.

Adaptation to the impact of $CaCO_3$ + $MgCO_3$ solution on the locomotion

After the ants had consumed the carbonates for 7 days, a trait affected by these compounds was again assessed (as it has previously been assessed) for examining if ants could adapt themselves to that impact, i.e. if the effect was of lower intensity. The trait here considered for making the present experiment was the ants' locomotion, i.e. their linear and their angular speeds. The distribution of obtained values (n = 40) were compared to the control ones using the non-parametric χ^2 test.

Habituation to the beneficial effect of $CaCO_3$ + $MgCO_3$ solution

A beneficial effect of calcium + magnesium carbonates on an ants' trait should have been assessed after several days of consumption for examining if ants became habituated to this beneficial effect. However, we found no beneficial effect of the carbonates solution on the different physiological and ethological traits we examined. The present study could thus not be performed.

Dependence on $CaCO_3$ + $MgCO_3$ consumption

We examined if ants developed some dependence on the calcium and magnesium carbonates consumption after they had these compounds at their disposal during 9 days. The used experimental protocol was similar to that employed in previous studies [5,23-25]. For each colony, 15 ants were transferred into a tray (15 cm × 7 cm × 5 cm) in which two tubes (length = 2.5 cm, diam. = 0.5 cm) had been deposited, one of them containing sugar water, the other one containing a sugar solution of calcium and magnesium carbonates, i.e. the solution used throughout all the present work (Figure 1L). The tube containing the carbonates solution was located on the right in one tray, and on the left in the other tray. The ants coming onto each tube were counted 15 times over 15 minutes and the counts corresponding to each kind of liquid were separately added. The counts obtained for each colony were also added. The sums were compared to the numbers expected if ants went randomly drinking each kind of provided liquid, using the non-parametric goodness of fit χ^2 test [26].

Decrease of the effect of $CaCO_3$ + $MgCO_3$ after their consumption was stopped

This decrease was examined, after the ants had consumed the carbonates solution during a total of 22 days, using an experimental protocol used in previous studies [5,25]. Some fresh solution of the carbonates was given to the ants 12 hours before the weaning time, and an assessment of the ants' sinuosity was made after these 12 hours, so just before the weaning time, i.e. at t = 0. Weaning began just after, when the solution of the carbonates was removed from the ants' tray and replaced by an ordinary aqueous solution of sugar. Since this time, the ants' sinuosity was quantified, about each two hours, as it had been before the ants consumed the carbonates (= control, i.e. while under normal diet), after they consumed them for one day, and after they consumed them for 8 days, except that only 20 instead of 40 ant's trajectories were analyzed for being able to make the assessments in the course of the experimentation. The distributions of the 20 values obtained after given time periods were compared to that obtained at t = 0 and to the control one (made of 40 values) using the non-parametric χ^2 test. Moreover, using Statistica V.10 software, a non parametric Kruskal-Wallis one-tailed test (K-W test) for multiple comparisons [26] was also used to compare the values of sinuosity corresponding to times ranging from t = 2h to t = 14h after weaning to either the control values (made of 20 values out of 40 in such a way that the median and quartiles were similar to those of the 40 values) or the values at the beginning of weaning (t = 0h), taking the two latter groups as control groups. A Bonferroni adjustment is incorporated. The numerical results are given in Table 6 and the decrease of the effects is graphically presented in the figure 2. The experiment ended when the ants' sinuosity became similar to that presented before consuming the carbonates solution.

Results and Discussion

Meat and sugar water consumption, general activity

Under calcium and magnesium carbonates diet, the ants consumed far less meat and sugar water and were slightly less active than while living under normal diet (Table 1). This was obvious to any observer and was statistically significant (meat, sugar water as well as activity: N = 6, T = -21, P = 0.016).

Days	Colonies	Sugar water diet			Sugar water + $CaCO_3$ and $MgCO_3$ diet		
		meat	sugar water	activity	meat	sugar water	activity
		Daily counts					
I	A	1 1 1 1 2 1	2 2 3 2 2 1	10 11 10 13 14 14	0 0 1 1 0 0	0 0 0 0 0 1	9 9 8 9 8 8
	B	0 0 1 1 0 0	1 1 1 1 0 2	10 11 11 12 12 11	1 0 0 0 0 1	0 0 0 1 0 0	8 8 9 7 8 8
II	A	2 2 2 2 2 3	5 6 4 4 4 5	13 12 13 13 12 13	1 1 0 1 0 0	0 0 1 0 0 0	10 11 11 14 13 13
	B	3 3 2 3 3 2	2 2 3 2 2 2	8 9 9 7 8 8	0 1 1 0 0 1	2 2 1 1 1 1	6 7 7 9 9 10
III	A	1 1 1 1 1 1	4 4 5 5 5 4	9 9 10 14 13 13	0 0 1 1 0 0	1 1 1 1 1 1	11 12 12 10 9 11
	B	2 2 3 2 3 3	3 3 2 3 3 2	8 8 9 9 10 10	1 1 1 0 0 0	0 0 1 0 0 1	6 7 8 7 8 7
IV	A	3 3 2 2 2 3	3 3 4 2 2 2	13 13 12 10 12 12	2 2 1 1 2 1	3 3 4 3 3 3	13 12 11 12 13 12
	B	3 3 3 2 2 2	1 2 2 2 2 1	12 12 13 9 12 12	2 1 1 1 1 1	1 1 1 1 1 1	9 9 8 11 12 11
V	A	3 4 4 3 3 2	4 4 5 4 4 3	12 11 12 13 13 12	0 0 0 1 1 0	1 0 0 0 0 0	10 10 9 11 11 12
	B	2 2 2 2 2 2	3 3 2 2 2 3	8 8 7 9 9 8	1 1 1 0 0 1	0 0 1 1 1 0	7 7 8 7 8 9
VI	A	1 1 1 1 1 1	5 5 6 3 4 4	13 14 14 14 14 15	0 0 0 0 0 0	2 2 3 2 2 3	16 14 14 13 14 14
	B	1 2 3 3 3 4	3 2 2 2 2 3	11 12 12 10 9 11	0 0 0 1 1 0	1 1 1 1 0 0	7 7 6 5 6 5
		Daily means					
I	A + B	0.75	1.50	11.58	0.25	0.17	8.25
II	A + B	2.42	3.42	10.42	0.50	0.75	10.00
III	A + B	1.75	3.58	10.17	0.42	0.67	9.00
IV	A + B	2.50	2.17	11.83	1.33	2.08	11.08
V	A + B	2.58	3.25	10.17	0.50	0.33	9.00
VI	A + B	1.83	3.42	12.33	0.17	1.50	10.08
		Average of daily means					
		2.47	2.89	11.08	0.53	0.92	10.08

Table 1. *Effect of calcium and magnesium carbonates on food consumption and general activity. Experimental details and statistics are given in the text. Briefly, the two carbonates largely decreased the ants' food consumption and slightly decreased their general activity.*

Linear and angular speed

Under calcium and magnesium carbonates diet, the ants moved more slowly and sinuously than while being under normal diet (Table 2, lines 1, 2). This was statistically significant (linear speed: $\chi^2 = 8.42$, df = 3, $0.02 < P < 0.05$; angular speed: $\chi 2 = 26.52$, df = 3, $P < 0.001$). After the ants consumed the two compounds for 7 days, it was examined if they became adapted to that impact of the compounds on their locomotion (see below, the paragraph dealing with adaptation).

Orientation to an alarm signal

While under normal diet, the ants oriented themselves very well towards an alarm signal. When they consumed calcium and magnesium carbonates, they did so less well (Table 2, line 3; Figure 1A). In fact, they often turned back before reaching the alarm signal, or deviated while approaching that signal, as if they feared being confronted to the signal. The difference of ants' orientation values according to their diet was significant: $\chi^2 = 10.94$, df = 2, $0.001 < P < 0.01$. A following experiment examined the impact of the two compounds on the ants' audacity (see below).

Trail following

This trait was affected by calcium and magnesium carbonates consumption (Table 2, line 4). While under normal diet, the ants followed a circular trail meanly along 12.5 arcs of 10 ang. deg., under the two compounds diet, they followed such a trail only along meanly 5 arcs of 10 ang. deg. (Figure 1B). This was statistically significant ($\chi^2 = 28.87$, df = 3, $P < 0.001$) and may be due to the impact of the carbonates on the ants' locomotion (see above).

Audacity

$CaCO_3$ + $MgCO_3$ consumption impacted this trait. Ants under normal diet were not very inclined to come onto the presented risky and unknown apparatus, but a few ones nevertheless did so. While consuming the two carbonates, the ants were still less inclined to come on the apparatus (Figure 1C). Very few ones were seen on it (Table 2, line 5), and the difference in the numbers of ants counted on the apparatus between the two kinds of diet was statistically significant (N = 5, T = -15, P = 0.031). The carbonates diet reduced thus the ants' straightforwardness, what was in agreement with a previous result (see above 'Orientation towards an alarm signal').

Tactile (pain) perception

Unexpectedly, this trait was somewhat impacted by the carbonates consumption (Table 2, line 6). Ants living under normal diet obviously perceived the uncomfortable character of the presented rough substrate and walked on it very slowly and sinuously (Figure 1D), touching the substrate in front of them with their antennae. The consumption of the carbonates decreased the ants' linear speed and increased their sinuosity (see above). Therefore, while consuming these compounds and if correctly perceiving the rough character of the substrate, the ants should walk on it slower and more sinuously than ants living under normal diet. They did not do so. On the contrary, they walked more quickly and less sinuously (Figure 1E), and this was highly significant (linear speed: $\chi^2 = 27.17$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 15.46$, df = 2, $P < 0.001$). The ants' tactile (pain) perception was thus reduced by the carbonates consumption.

Traits	Sugar water diet	Sugar water+ $CaCO_3$ and $MgCO_3$ diet
Linear speed (mm/s)	12.5 (11.4 - 14.4)	10.1 (8.5 - 12.4)
Angular speed (ang.deg./cm)	120 (99 - 145)	154 (144 - 175)
Orientation (ang. deg.)	42.0 (32.3 - 68.4)	59.2 (44.9 - 70.9)
Trail following (n° arcs)	12.5 (8.0 - 18.0)	5.0 (4.0 - 8.0)
Audacity (n° ants)	1.70 [1 - 3]	0.70 [0 - 1]
Tactile (pain) perception:		
Linear speed (mm/s)	5.6 (4.8 - 6.5)	8.4 (7.7 - 9.3)
Angular speed (ang.deg./cm)	276 (255 - 299)	191 (171 - 217)

Table 2: *Effects of calcium and magnesium carbonates on six physiological and ethological traits. Consuming these carbonates led to a decrease of linear speed, orientation ability, trail following, audacity, tactile (pain) perception, and an increase of sinuosity. Statistics and experimental details are given in the text. mm/s = millimeter per second; ang.deg.: Angular Degree; ang.deg./cm: Angular Degree/Centimeter; n°: Number. The table gives the median (and quartiles) or the mean [and the extremes] of the obtained experimental values.*

Brood caring

This trait was not impacted by the carbonates consumption (Table 3, line 1). Ants consuming them, just like those living under normal diet, quickly came near the larvae and the nymphs experimentally removed from the nest, took care of them, and replaced them in the nest as soon as they could. The difference of not re-entered larvae between the two kinds of diet was not significant (N = 3, NS). However, when attentively looking to ants transporting a larva, a qualitative difference could be noted between ants consuming or not the carbonates. Under normal diet, the ants hold up the transported larva; under the carbonates diet, they did not so highly held the transported larva, which was thus at the limit of touching the ground (Figure 1F, 1G). This difference between ants living under one or the other kind of diet was in agreement with that pointed out concerning the ants' locomotion (see above 'Linear and angular speed'). In any way, $CaCO_3$ + $MgCO_3$ consumption did not impact the ants' relationship with their brood, so very probably their social relationship, a presumption checked in the following experiment (see just below).

Aggressiveness against nestmates and aliens

These two traits were not affected by the two carbonates consumption (Table 3, lines 2 and 3). Ants under normal as well as under $CaCO_3$ + $MgCO_3$ diet never aggressed their nestmates (Figure 1H). There was no statistical difference between the behavior of ants under one or the other kind of diet ($\chi^2 = 3.52$, df = 2, $0.10 < P < 0.20$). In the same way, ants under normal as well as under the carbonates diet always aggressed alien ants (Figure 1I). There was again no statistical difference between the behavior of ants under one or the other kind of diet ($\chi^2 = 3.41$, df = 4, $0.30 < P < 0.50$). It can thus be concluded that the carbonates consumption did not affect the ants' social relationship, as already deduced from the previous experiment (see just above).

Cognition

This trait appeared, at first sight, to be impacted by $CaCO_3$ + $MgCO_3$ consumption (Table 3, line 4). Under the carbonates diet, the ants were far less inclined to move through the twists and turns paths than when living under normal diet. This was statistically significant (small area in front of the difficult path: N = 7, T = +26.5, P = 0.019; large area beyond the difficult path: N = 5, T =-15, P = 0.031). Ants consuming the carbonates hesitated to enter the difficult path, and if doing so, they often came back on their way. They presented thus a reduction of forwardness (a fact already revealed in a previous experiment, see above 'Audacity'), and perhaps also a slight decrease of their cognitive ability. The impact of $CaCO_3$ + $MgCO_3$ diet on the ants' audacity and cognition was again examined in the two following experiments (see just below).

Escaping ability

This trait was impacted by the carbonates consumption (Table 3, line 5). Under normal diet, 10 ants among 12 could escape from the enclosure while under the carbonates diet, only 3 ones could escape. The difference of behavior between ants under the two kinds of diet was significant (for ants escaped as well as for those still enclosed: N = 6, T = - or + 21, P = 0.016). Ants consuming the carbonates came in front of the exit and hesitated in moving through it (Figure 1J). They went away, stopped, and then moved again slowly inside the enclosure. Only a few ones went out through the enclosure. The ants consuming the carbonates failed in escaping not because they stressed (very stressed individuals failed in escaping [13]), but because they presented some reduction of straightforwardness (a deduction already made on basis of two previous experiments, see above 'Audacity' and 'Cognition'), and perhaps some decrease of their cognition or cerebral activity, a hypothesis examined in the following experiment (see just below).

Traits	Sugar Water Diet	Sugar Water+ $CaCO_3$+ $MgCO_3$
Brood caring: numbers of larvae not re-entered in the nest over 10 minutes	time: 30s 2 4 6 8 10 n° : 10 8 6 2 1 0	time: 30s 2 4 6 8 10 n° : 10 8 6 4 2 0
Aggressiveness against nestmates	Levels 0 1 2 3 4 var 'a' n° 77 47 11 0 0 0.09	levels 0 1 2 3 4 var 'a' n° 68 70 15 0 0 0.11
Aggressiveness against aliens	Levels 0 1 2 3 4 var 'a' n° 9 36 57 55 34 3.25	Levels 0 1 2 3 4 var 'a' n° 10 25 49 39 41 3.69
Cognition: ants in front of and beyond twists and turns in the course of 12 minutes	t n° in front n° beyond 30s 30 0 2 24 0 4 23 1 6 21 2 8 19 4 10 18 4 12 17 5	t n° in front n° beyond 30s 29 0 2 27 0 4 27 0 6 24 0 8 22 0 10 21 0 12 18 1
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 minutes	t: 30s 2 4 6 8 10 12 n° in 12 10 8 6 4 3 2 n° out: 0 2 4 6 8 9 10 variable = 10/12 = 0.83	t: 30s 2 4 6 8 10 12 n° in: 12 12 12 11 10 9 9 n° out: 0 0 0 1 2 3 3 variable = 3/12 = 0.25

Table 3: *Effects of calcium and magnesium carbonates on five ethological and physiological traits. Details and statistical results can be found in the text. Consuming the carbonates did not affect the ants' brood caring, nor their aggressiveness, and thus, not their social relationship, but impacted their cognition and escape ability. s: Second; n°: Number.*

Figure 1: *A few views of the experiments. A: Ants under $CaCO_3$+ $MgCO_3$ diet poorly orienting themselves towards a tied congener emitting its alarm pheromone. B: Two ants under $CaCO_3$+ $MgCO_3$ diet, one following a circular trail, one departing from it. C: Two ants under $CaCO_3$+ $MgCO_3$ diet not at all inclined in coming onto the presented unknown apparatus. D: Trajectories made in 5 s by ants under normal diet on a rough substrate; the trajectories are very short (~ 4 mm) and sinuous. E: Trajectories made in 5s by ants under $CaCO_3$+ $MgCO_3$ diet on a rough substrate; the trajectories are longer (~ 6 mm) and thus less sinuous than those of ants under normal diet. F: An ant under normal diet holding and lifting a nymph, and thus its head. G: An ant under $CaCO_3$+ $MgCO_3$ diet transporting a larva, not sufficiently lifting it. H: Two nestmates under $CaCO_3$+ $MgCO_3$ diet presenting no aggressive reaction against one another. I: An ant under $CaCO_3$+ $MgCO_3$ diet stinging an alien ant. J: Two ants under $CaCO_3$+ MgO_3 diet walking in front of the exit of an enclosure and not escaping. K: An ant under $CaCO_3$+ $MgCO_3$ diet, trained to a hollow green cube, giving the correct response during a test in a Y-apparatus provided with this cue in one of its branch. L: Ants under $CaCO_3$+ $MgCO_3$ diet, in presence of sugar water containing these carbonates (red mark) and of sugar water free of carbonates, preferentially drinking the latter solution. s: Second; mm: Millimeter.*

Visual and olfactory conditioning and memory

These cognitive abilities appeared to be affected by $CaCO_3$ + $MgCO_3$ consumption (Table 4).

Concerning the ants' visual conditioning, ants living under normal diet reached a score of 70% after 7 training hours and a score of 80% after 24 - 72 training hours. While consuming calcium and magnesium carbonates, the ants only reached a score of 60% after 7 training hours, of 65% after 24 training hours, and of 75% after 72 training hours. The difference of conditioning ability between ants living under one or the other kind of diet was significant: N = 6, T = -21, P = 0.016. After removal of the visual cue, ants living under normal diet still presented a conditioning scores of 80% after 7 hours and of 70% after 72 hours. While consuming calcium and magnesium carbonates, the ants presented the low scores of 65% after 7 hours and of 50% after 72 hours. They thus rapidly entirely lost their visual conditioning. The difference of visual memory between ants living under one or the other kind of diet was statistically significant: N = 6, T = -21, P = 0.016.

Concerning the ants' olfactory conditioning, ants living under normal diet reached a score of 70% after 7 training hours and a score of 90% after 48 - 72 training hours. Ants consuming calcium and magnesium carbonates only reached a score of 55% after 7 training hours and of 65% after 72 training hours. Once more, the difference of conditioning ability between ants living under one or the other kind of diet was significant: N = 6, T = -21, P = 0.016. After removal of the olfactory cue (basilica), the ants living under normal diet presented the high score of 90% after 7 hours and still a score of 80% after 72 hours. While consuming calcium and magnesium carbonates, the ants presented a score of 50% after 7 hours and again of 50% after 72 hours. They thus lost their weak olfactory conditioning very quickly, in a few hours. The difference of olfactory memory between ants consuming or not consuming calcium and magnesium carbonates was statistically significant: N = 6, P = -21, P = 0.016.

The carbonates diet appeared thus to severely affect the ants' learning capability, so their short-term memory, as well as their middle term memory, an observation we did not expect. Moreover, though we could have no statistical confirmation, it seemed that this impact of calcium and magnesium carbonates on the ants' learning and memory increased over their consumption (Table 4: compare the 12 last lines, i.e. the second period of the experiment, with the 12 first lines, i.e. the first period of the experiment).

Traits Time (h)	Normal diet*		$CaCO_3$ + $MgCO_3$ diet		
	Colony C	%	Colony A	Colony B	%
Visual conditioning					
7	7	70	6	6	60
24	8	80	7	6	65
31	8	80	8	6	70
48	8	80	7	7	70
55	8	80	8	7	75
72	8	80	7	8	75
Visual memory					
7	8	80	7	6	65
24	7	70	5	7	60
31	8	80	5	6	55
48	7	70	6	5	55
55	7	70	5	6	55
72	7	70	5	5	50
Olfactory conditioning					
7	7	70	5	6	55
24	8	80	5	4	45
31	8	80	6	6	60
48	9	90	5	7	60
55	9	90	7	4	55
72	9	90	6	7	65
Olfactory memory					
7	9	90	4	6	50
24	8	80	5	6	55
31	8	80	5	6	55
48	7	70	4	5	45
55	8	80	5	5	50
72	8	80	5	5	50

Table 4: *Effect of calcium and magnesium carbonates on ants' conditioning ability and memory. These carbonates decreased the examined ants' cognitive traits. This impact may become more pronounced over time: experimental time began at line 1 and ended at line 24, and the impact of the medicine on the ants' examined traits increased over the running time. More information is given in the text. %: Percentage. *: Result obtained in a previous study [22].*

Adaptation to the impact of $CaCO_3$ + $MgCO_3$ on the locomotion

No adaptation to the impact of $CaCO_3$ + $MgCO_3$ consumption on the ants' locomotion was observed (Table 5). After 7 days under these carbonates diet, the ants still walked slower and more sinuously than when living under normal diet. This was statistically significant; linear speed: χ^2 = 27.35, df = 3, P < 0.001; angular speed: χ^2 = 36.89, df = 3, P < 0.001. It can even be presumed that the impact of the carbonates on the ants' locomotion increased in the course of time since the values of χ^2 obtained after 7 days of consumption were higher than those obtained after 1 day of consumption (linear speed: χ^2 = 27.35 vs 8.42; angular speed: χ^2 = 36.89 vs 26.52). Such a lack of adaptation to the adverse effects of a product is not in favor of its consumption.

Traits	Normal diet	$CaCO_3$ + $MgCO_3$ since one day	$CaCO_3$ + $MgCO_3$ since 7 days
Linear speed (mm/sec)	12.5 (11.4 - 14.4)	10.1 (8.5 - 12.4)	9.1 (7.9 - 10.6)
Angular speed (ang.deg./cm)	120 (99 - 145)	154 (144 - 175)	189 (167 - 228)

Table 5: *Ants' adaptation to the impact of calcium and magnesium carbonates on their locomotion. Ants developed no adaptation to the impact of these carbonates on their locomotion; their linear and angular speeds were still (and even more) different from the normal one after 7 days of these carbonates consumption. More information is given in the text. mm/s: Millimeter Per Second; ang.deg./cm: Angular Degree/Centimeter.*

Habituation to beneficial effects of $CaCO_3$ and $MgCO_3$

No beneficial effect (except a possible decrease of the digestive track acidity) having been noted for these two carbonates, habituation could not be investigated in the course of our study on ants as models.

Dependence on $CaCO_3$ + $MgCO_3$ consumption

Ants developed no dependence on the two examined carbonates consumption. In presence of sugar water containing these carbonates and of sugar water free of them, 10 ants of colony A went drinking the former solution and 13 ants the latter solution, while 6 ants of colony B went drinking the former solution and 16 ants the latter solution. A total of 20 ants chose the solution free of calcium and magnesium carbonates, and 16 ones the solution containing these carbonates. The difference did not statistically differ from that resulting from a random choice of the two liquids (χ^2 = 1.37, df = 1, 0.20 < P < 0.30). It can thus be concluded that ants developed no dependence on calcium and magnesium carbonates, and even preferred not consuming these compounds, what is in favor of the drug use. A lack of dependence occurs when the effect of the product slowly decreases after weaning, a fact the following experiment examined (see just below).

Decrease of the effect of $CaCO_3$ + $MgCO_3$ after their consumption was stopped

The results of this study are presented numerically in table 6 and graphically in figure 2. Briefly, the effects of $CaCO_3$ + $MgCO_3$ on the ants' locomotion slowly decreased over time after weaning. In detail, from 2 to 6 hours after weaning, the impact of the carbonates was still similar to the initial one (vs t = 0: 2h: χ^2 = 2.60, df = 2, 0.20 < P < 0.30; 4h: χ^2 = 2.20, df = 2, 0.30 < P < 0.50; 6h: χ^2 = 2.47, df = 2, 0.20 < P < 0.30) and the ants' sinuosity highly differed from their control one (vs control: 2h: χ^2 = 28.65, df = 3, P < 0.001; 4h: χ^2 = 27.20, df = 3, P < 0.001; 6h: χ^2 = 22.79, df = 3, P < 0.001). Eight hours after weaning, this impact was weaker since the ants' sinuosity was statistically different from the initial one (vs t = 0: χ^2 = 9.29, df = 2, P ~ 0.01), but did not yet equal the control one (vs control: χ^2 = 15.21, df = 3, 0.001 < P < 0.01). Ten hours after weaning, the impact of the carbonates was very weak. Indeed, the ants' sinuosity largely differed from the initial one (vs t = 0: χ^2 = 24.44, df = 2, P < 0.001) and became not statistically different from the control one (vs control: χ^2 = 6.91, df = 3, 0.05 < P < 0.10). Twelve hours after weaning, the ants' sinuosity of course statistically differed from the initial one (vs t = 0: χ^2 = 20.13, df = 2, P < 0.001) and was, this time, statistically similar to the control one (vs control: χ^2 = 4.65, df = 3, P = 0.20), even if the obtained sinuosity mean value was not yet identical to the control one. Fourteen hours after weaning, the ants walked exactly as before consuming the two carbonates (vs t = 0: χ^2 = 25.25, df = 2, P < 0.001; vs control: χ^2 = 0.11, df = 2, P ~ 0.95) (Table 6). We then also observed that the ants came eating meat and drinking sugar water as usual, were active as usual and no longer hesitated in coming onto unknown elements. The numerical results obtained in the course of this study of the decrease of the effects of calcium and magnesium carbonates after weaning were also statistically analyzed using one-tailed non-parametric Kruskal-Wallis ANOVA for multiple comparisons. These results, given in table 6, were fully in agreement with the χ^2 tests analysis. It can thus be concluded that the effects of the carbonates on the health vanished significantly in 10 hours after weaning and completely in a total of 14 hours.

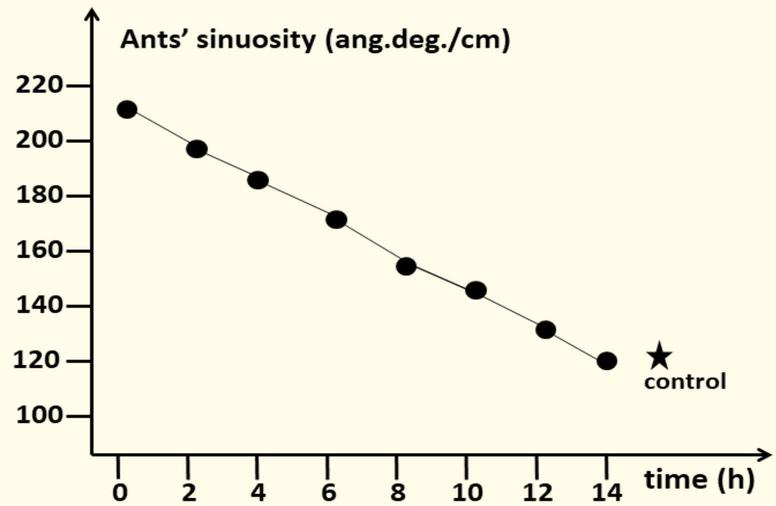

Figure 2: *Decrease of the effect of calcium and magnesium carbonates on the ants' sinuosity of movement after the consumption of these substances was stopped. Details are given in the text; numerical values can be found in table 6. Such an effect slowly and linearly decreased over time, and totally vanished in 14 hours. ang.deg./cm: Angular Degrees/Centimeter; h: Hour.*

Experiments and times	Ants' sinuosity ang.deg./cm	Statistics			
		vs control		vs t = 0h	
		χ^2	K - W	χ^2	K - W
Control	120 (99 - 145)				
After 1 day of consumption	154 (144 - 175)				
After 8 days of consumption	189 (167 - 228)				
After 22 days: weaning.					
Time after weaning:					
t = 0h	211 (202 - 235)	P < 0.001	P < 0.001		
t = 2h	198 (150 - 230)	P < 0.001	P < 0.001	0.20 < P < 0.30	P = 0.35
t = 4h	185 (151 - 223)	P < 0.001	P < 0.001	0.30 < P < 0.50	P = 0.52
t = 6h	178 (154 - 205)	P < 0.001	P < 0.001	0.20 < P < 0.30	P = 0.12
t = 8h	155 (141 - 173)	P < 0.01	P = 0.016	P~0.01	P = 0.004
t = 10h	149 (136 - 169)	0.05 < P < 0.10	P = 0.13	P < 0.001	P < 0.001
t = 12h	132 (114 - 154)	P = 0.20	P = 1	P < 0.001	P < 0.001
t = 14h	119 (113 - 125)	P ~0.95	P = 1	P < 0.001	P < 0.001

Table 6: *Decrease of the effect of calcium and magnesium carbonates on the ants' sinuosity of movement, after the consumption of these carbonates was stopped. This decrease was slow, linear (see details in the text), and the effects of the carbonates vanished in a total of 10 to 14 hours. Non - parametric χ^2 test. 'K-W: one - tailed non - parametric Kruskal - Wallis ANOVA for multiple comparisons, taking either 'control' or 't = 0h' as control groups. The decrease is graphically shown in figure 2. t: Time; h: Hour.*

This decrease occurred according to a linear function, the angular coefficient (the mean of the successive obtained angular coefficients) equaling 13.1. It obeyed thus to the function:

$E_t = E_{t=0} - 13.1\ t$

(with E = effect, t = time, $E_{t=0}$ = initial effect, E_t = effect at a time 't' over the decrease)

Such a slow linear decrease of the effect of the two carbonates after weaning is in accordance with the absence of dependence on these carbonates consumption, a fact always observed all along our studies of the effects of products used by humans [25,28].

Comparison with medicinal and pharmaceutical observations and researches

Having found that aluminum hydroxide, used for decreasing the stomach acidity, has several adverse effects [5] and being so in agreement with other researchers' results [1-4], we here examined the potential effects of another drug having the same medicinal efficiency, a mixture of calcium and magnesium carbonates, using the same procedure. Unexpectedly, we discovered that this product decreased the ants' food consumption, general activity, orientation capability, trail following, audacity, tactile perception, cognition, escaping ability, learning and memory. The ants' social relationship was not impacted, but these insects never adapted themselves to the adverse effects of the carbonates which even increased over time. No dependence on the carbonates consumption occurred, and the effects of these compounds slowly decreased over time after weaning, fully vanishing in a total of 14 hours. Being rather surprised by our findings, we attentively looked to information available on calcium and magnesium carbonates use for caring of patients. No research work could be found, and only detailed instructions of use joined to the package of medicines (Rennie®, Magnésie Plus®), as well as internet sites [29-32], brought us the wanted information. Here below, we relate this information relative to humans, ordering the observed effects according their physiological implications.

Calcium and magnesium carbonates lead to 1) a decrease of the acidity of the digestive track, 2) an increase of the amount of calcium and of magnesium in the blood, 3) a loss of the required acidic-basic balance inside the body. These three consequences of calcium and magnesium carbonates consumption induce several health problems. The most observed ones are: a large decrease of the appetite, some decrease of the gustative perception, a reduction of the intestinal absorption of several important substances, some muscular weakness, tiredness, loss of strength, neuromuscular problems, confusion, impact on the nervous system, decrease of the reflexes, psychic troubles. We were surprised that medicines with so many adverse effects can easily be found in any drugstore and be consumed with no warming, and by the concordance with what we observed on the experimented ants. Indeed, comparing with the above list of pharmacologists' observations, the ants presented a decrease of food consumption, a decrease of tactile perception, some deficiency in holding the larvae, some decrease of general activity, locomotion disorders, a decrease of the audacity, a decrease of the orientation and of the trail following, a decrease of the escaping behavior, a large decrease of the conditioning capability, and a decrease of short-term and middle-term memory. No adaptation to these effects occurred. We observed no change in the social relationship, as well as no dependence on the examined product.

We previously studied on ants the adverse effects of aluminum hydroxide, the other compound used for treating persons suffering from stomach acidity [5]. We found that it has as many adverse effects as the calcium and magnesium carbonates we here studied, though similarly not affecting the ants' social relationship. Our findings on the effects of aluminum hydroxide and Ca + Mg carbonates on ants agreed with observations made on humans [1-4].

Conclusion

We used ants as test animals for examining the effects of $CaCO_3$ + $MgCO_3$, as we previously examined those of aluminum hydroxide. We conclude that calcium and magnesium carbonates as well as aluminum hydroxide have adverse physiological and behavioral effects. Because of these unwanted effects, these products should be used as gastric antacids only momentarily, i.e. never for a long time period. For a long time period or a permanent use, an alternative should be researched to help patients. In a future work, we shall therefore examine the effects of green clay, a natural product which is very efficient in reducing heartburn.

Conflict of Interest

We affirm having no conflict of interest as for the use of the examined product. We receive no money for making our research. We are independent researchers, studying ants' ethology and physiology.

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29. Calcium and Magnesium Carbonates Tablets
30. Drug Index A to Z
31. Calcium Carbonate Tablets (Antacid)
32. Food and Drink

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