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Significance of milk pH in Newborn Infants

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Summary

Bottle-fed infants do not gain weight as rapidly as breast-fed babies during the first week of life. This weight lag can be corrected by the addition of a small amount of alkali (sodium bicarbonate or trometamol) to the feeds. The alkali corrects the acidity of cow's milk which now assumes some of the properties of human breast milk. It has a bacteriostatic effect on specific *Escherichia coli* in vitro, and in infants it produces a stool with a preponderance of lactobacilli over *E. coli* organisms. When alkali is removed from the milk there is a decrease in the weight of an infant and the stools contain excessive numbers of *E. coli* bacteria.

A pH-corrected milk appears to be more physiological than unaltered cow's milk and may provide some protection against gastroenteritis in early life. Its bacteriostatic effect on specific *E. coli* may be of practical significance in feed preparations where terminal sterilization and refrigeration are not available. The study was conducted during the week after birth, and no conclusions are derived for older infants. The long-term effects of trometamol are unknown. No recommendation can be given for the addition of sodium bicarbonate to milks containing a higher content of sodium.

Introduction

Cow's milk differs from human milk in many respects. It has a high protein and phosphate content and produces a firm stool with a preponderance of *Escherichia coli* organisms. Breast milk, with its high lactose, low phosphate, and low protein load, produces a stool with a relatively low *E. coli* count (Bullen and Willis, 1971). In addition, it affords protection against gastroenteritis.

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A consistent difference has been noted in the weight patterns of normal full-term breast-fed and bottle-fed infants at Mowbray Maternity Hospital, Cape Town. Breast-fed babies regain birth weight a day earlier than bottle-fed infants despite their larger initial weight loss (Table I). The delayed weight gain in bottle-fed babies can be corrected or prevented by the addition of sodium bicarbonate to the milk. A pattern of delayed gain in weight has been described in premature infants who manifest late metabolic acidosis (Kilderberg, 1964). Sodium bicarbonate given by mouth produced a gain in weight as well as a correction of the acidosis. The full-term bottle-fed infants did not show signs of acidosis during the phase of growth lag. Nevertheless, they gained weight within 24 hours after the addition of alkali to feeds. Further investigations indicated that cow's milk was more acid than human breast milk.

This study was undertaken to determine the significance of these observations. It is concerned with the effects of a pH-corrected cow's milk on the early growth pattern of newborn infants and on the nature of their stool flora. The investigations were conducted on similar groups of breast-fed and bottle-fed babies.

Material and Methods

Breast Milk pH.—The pH was measured daily for seven days on the colostrum and milk of 92 lactating mothers. Milk was expressed directly into a sterile test tube at a fixed time each day. This was done before a feed. In 10 cases a second sample was collected under anaerobic conditions in a capillary tube. The ends were immediately sealed with wax. The pH was measured within five minutes by the Astrup technique, using a capillary electrode (Radiometer G297/G2).

Cow's Milk pH.—Twenty-four milks of varying strengths and makes were reconstituted with water (pH 7·80) to a volume of 75 ml. They were then sterilized at 110°C at 15-lb (6·8-kg) pressure for 10 minutes and then refrigerated. Each feed was re-warmed to 38°C and the pH measurements were done in triplicate. These milks were prepared on five separate occasions for pH readings.

Milk Titration.—Titratable acidity was determined on all samples submitted for pH measurement. A 6-ml volume of

TABLE I—*Birth Weight and Percentage of Birth Weight of 50 Breast-fed and 50 Bottle-fed Full-term Infants. Mean ± S.D.*

	Birth Weight (in grammes)	Days after birth (% of Birth Weight)					
		3	4	5	6	7	8
Human breast	3,304 ± 352	97·3 ± 2·1	98·3 ± 2·5	99·3 ± 2·7	100·2 ± 3·0	101·5 ± 3·9	—
Cow's milk	3,345 ± 386	97·9 ± 2·2	98·5 ± 2·8	98·9 ± 3·2	99·3 ± 4·1	100·0 ± 4·5	100·9 ± 4·7

sterile cow's milk and the same volume of fresh breast milk was titrated with 4·2% sodium bicarbonate to a pH of 7·40. End-point titration was determined on an automatic titrating unit (Radiometer ABU 11, TT 11, PMG 27). Titration was repeated by using the alkaline solution trometamol (Tham).

Viable E. coli *Counts in Milk.*—Bacteriological cultures were performed on 20 samples of reconstituted sterile cow's milk and 20 samples of fresh breast milk. A 6-ml sample of milk was inoculated with about 2 by 10^2 specific *E. coli* O111/B4 organisms. Viable counts were done on blood agar by the drop method (Miles *et al.*, 1938) after 1, 3, 4, 6, and 7 hours of incubation at 37°C. All counts were done in triplicate. Similar cultures were repeated on titrated milk samples.

Infant Study.—The weights of 100 consecutive full-term infants were recorded for the first week of life. Fifty infants were breast-fed and 50 were bottle-fed. Their daily weight variations were expressed as a percentage of birth weight. The following small groups of infants were studied in detail: group 1, 10 normal full-term breast-fed babies; group 2, 10 normal bottle-fed infants; group 3, 10 bottle-fed infants given sodium bicarbonate in feeds; and group 4, 10 bottle-fed babies given trometamol in feeds.

Weights.—The infants in each group were undressed and weighed at a fixed time each day before feeding. A dial scale (Salter model 40A) was used for this purpose.

Milk.—A humanized milk (Similac, Ross Laboratories) was given to all bottle-fed infants. The initial mean milk pH of 6·70 was raised to about 7·20 in groups 3 and 4. This was done by adding 1 mEq sodium bicarbonate to each feed of the infants in group 3, and 2 ml trometamol to the feeds of infants in group 4. Infants received their particular feed within three hours of birth. Bottle-fed babies were offered a fixed volume of 100 ml of milk. The amount ingested at each feed was noted and charted, and a strict intake and output chart was kept for each baby. The alkali additives were removed from the milks on the seventh day.

Acid Base and Serum Sodium.—Arterialized blood was collected from the heel of each bottle-fed infant on the fifth day of life. Acid base was measured immediately by using the Astrup technique. Serum sodium was determined on non-heparinized blood by flame photometry. Every attempt was made to avoid investigations not in the interest of the infant. Blood was not taken from the group of breast-fed babies. The nature of the study was explained to each mother and her consent obtained for all investigations.

Stools.—A fresh stool specimen was collected daily from each infant. The stool was placed in a clean plastic carton and taken immediately to the laboratory.

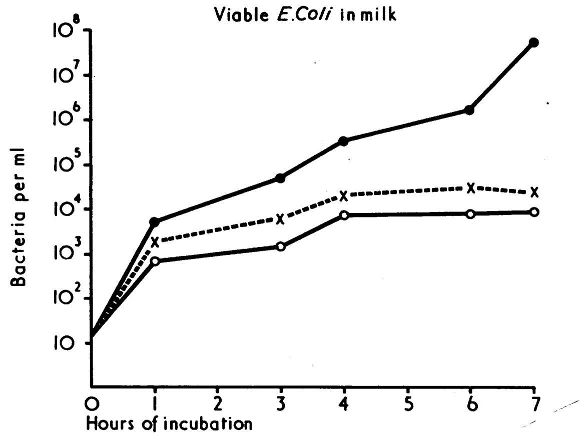
pH and Titration.—A 1-g sample of stool was emulsified in 20 ml normal saline. The pH of this solution was measured and it was then automatically titrated with 10N lactic acid to a pH of 4·5.

Quantitative Bacterial Counts.—A fresh sample of stool weighing 0·10 g was emulsified with 100 ml saline. Tenfold dilutions from 10^{-3} to 10^{-7} were prepared in normal saline. Bacterial counts were made from these dilutions on MacConkey and tomato juice agar plates by the drop method (Miles *et al.*, 1938). *E. coli* organisms were counted on MacConkey plates after 24 hours' aerobic incubation at 37°C. Lactobacilli were counted on tomato juice agar after 36 hours' anaerobic incubation at 37°C in 10% carbon dioxide. *E. coli* were identified as lactose fermenters on MacConkey's medium; lactobacilli were identified by colony appearance on tomato juice agar and by morphology on Gram stain. All investigations were subjected to statistical analysis, using the Kruskal Wallis one-way analysis of variance by ranks.

Results

MILK

pH and Titration.—Human breast milk had a mean pH of 7·29 (S.D. ± 0·19) with the range 6·80-7·70 for the week after birth. Little daily variation occurred, and no difference was observed in the pH of milk collected under anaerobic conditions. The milk required an average of 0·07 mEq (S.D. ± 0·04) sodium bicarbonate for titration. Reconstituted cow's milk had a mean



Specific *E. coli* 0111/B4 counts in milk during seven hours of incubation. O = Breast milk. ● = Cow's milk. X = Cow's milk with alkali (bicarbonate and trometamol).

TABLE II—*Specific* E. coli *0111/B4 Counts in Milk. Mean ± S.D.*

	Incubation Period (Organisms/ml)				
	1 Hour	3 Hours	4 Hours	6 Hours	7 Hours
Human breast	$8 \times 10^2 \pm 1 \times 10^3$	$2 \times 10^3 \pm 4 \times 10^3$	$9 \times 10^3 \pm 2 \times 10^3$	$9 \times 10^3 \pm 1.3 \times 10^3$	$8 \times 10^3 \pm 1.2 \times 10^3$
Cow's milk	$7 \times 10^3 \pm 1.2 \times 10^3$	$7 \times 10^4 \pm 2.4 \times 10^4$	$5 \times 10^5 \pm 2.4 \times 10^5$	$6 \times 10^6 \pm 2.6 \times 10^6$	$6.6 \times 10^7 \pm 3 \times 10^7$
Cow's milk + bicarbonate	$3.3 \times 10^3 \pm 1.6 \times 10^3$	$7.6 \times 10^3 \pm 1.4 \times 10^3$	$1.4 \times 10^4 \pm 7.1 \times 10^3$	$1.9 \times 10^4 \pm 8.3 \times 10^3$	$1.8 \times 10^4 \pm 8.6 \times 10^3$
Cow's milk + trometamol	$2.5 \times 10^3 \pm 1.0 \times 10^3$	$7.6 \times 10^3 \pm 2.0 \times 10^3$	$1.7 \times 10^4 \pm 9.0 \times 10^3$	$3.2 \times 10^4 \pm 9.0 \times 10^3$	$2.8 \times 10^4 \pm 8.8 \times 10^3$

Bacterial growth (three to seven hours' incubation) in unaltered cow's milk differs significantly ($P < 0.0005$) from that in human breast milk or cow's milk with alkali additives.

TABLE III—*Gestation and Weights of the 40 Infants in the Study Groups. Mean ± S.D.*

Group	Gestation (Weeks)	Birth Weight (in grammes)	Days after Birth (Weight in Grammes)							
			3	4	5	6	7	8	9	10
1	40·0 ± 0·4	3,158 ± 475	3,044 ± 464	3,057 ± 407	3,115 ± 403	3,161 ± 425	3,196 ± 405	—	—	—
2	39·7 ± 0·5	3,144 ± 356	3,083 ± 386	3,087 ± 371	3,090 ± 382	3,088 ± 409	3,125 ± 396	3,140 ± 377	3,171 ± 374	3,185 ± 382
3	40·0 ± 0·8	3,176 ± 372	3,120 ± 381	3,155 ± 359	3,192 ± 365	3,230 ± 368	3,246 ± 368	3,113 ± 331	3,194 ± 383	3,211 ± 354
4	40·0 ± 0·3	3,244 ± 394	3,144 ± 365	3,187 ± 394	3,208 ± 360	3,208 ± 378	3,245 ± 405	3,230 ± 454	3,277 ± 370	3,307 ± 390

Group 1, Breast fed (10 infants). Group 2, Bottle fed (10 infants). Group 3, Bicarbonate added to milk for seven days (10 infants). Group 4, trometamol added to milk for seven days (10 infants).

pH of 6·51 with a range from 5·60 to 6·95 depending on the particular brand and its dilution. A larger amount of sodium bicarbonate (average 0·64 mEq) was necessary for end-point titration.

Viable E. coli *Counts*.—Breast milk remained relatively bacteriostatic over a seven-hour period (see Chart). *E. coli* counts averaged 8×10^2 organisms/ml at one hour and thereafter the mean count did not exceed 9×10^3 (Table II). Reconstituted cow's milk averaged 7×10^3 organisms/ml at one hour and a mean $6{\cdot}6 \times 10^7$ at seven hours. Once alkali had been added to the milk the growth of bacteria was discouraged. Milks with sodium bicarbonate averaged *E. coli* counts of $3{\cdot}3 \times 10^3$ at one hour and reached a mean of $1{\cdot}8 \times 10^4$ at seven hours. Those with trometamol ranged from a mean $2{\cdot}5 \times 10^3$ (one hour) to $2{\cdot}8 \times 10^4$ at seven hours (Table II).

INFANTS

Weights.—Breast-fed babies lost a mean 2·7% of their birth weight by day 3 (Table I) and regained this weight 5·7 days after birth. Bottle-fed babies had an average weight loss of 2% and reached birth weight at a mean seven days of age. Weight differences in each group were not statistically significant, but the time taken to regain birth weight showed a significant variation between these groups ($P > 0{\cdot}05$). Similar weight patterns were seen in all groups and all had comparable weights and gestational ages (Table III). Breast-fed infants (group 1) had regained birth weight by 5·9 days of age, whereas bottle-fed infants (group 2) reached this weight at a mean 8·7 days of age. Infants fed an alkali (groups 3 and 4) had a weight pattern which resembled that in group 1. Those on bicarbonate (group 3) had regained birth weight by 5 days of age, while the trometamol group (group 4) regained this weight at a mean seven days after birth. In group 3, growth remained steady until 24 hours after the removal of alkali. At this stage (eighth day of life) a mean weight loss of 2% occurred, and thereafter weight increased up to the tenth day of age. Group 4 infants showed less striking growth arrest after the removal of alkali from the milk. Weight differences among these small groups were not statistically significant. The time taken to regain birth weight showed a significant difference in groups 1 and 2, and in groups 2 and 3 ($P > 0{\cdot}05$).

Fluids.—Adequate fluid intake was recorded in all bottle-fed infants for the first seven days of life. Those not receiving alkali averaged 512 ml a day (162 ml/kg birth weight) while the bicarbonate group ingested a mean 488 ml a day (153 ml/kg birth weight), and the trometamol group received a mean 539 ml a day (165 ml/kg birth weight).

Acid Base and Serum Sodium.—Normal acid base values were present in all bottle-fed babies. Those in group 2 had a mean pH of 7·40 (S.D. ± 0·02), a Pco_2 of 41·0 mm Hg (S.D. ± 3·0), and a base excess of + 0·7 mEq. The values for group 3 were: mean pH 7·40 (S.D. ± 0·02), Pco_2 43·7 mm Hg (S.D. ± 3·5), base excess + 2·0 mEq; and for group 4 the values were: mean pH 7·40 (S.D. ± 0·02), Pco_2 42·2 mm Hg (S.D. ± 2·7), and base excess + 1·1 mEq. Serum sodium averaged 141 mEq/l. (S.D. ± 2·8) in group 2, 142 mEq/l. (S.D. ± 5·0) in group 3, and 140 mEq/l. (S.D. ± 4·8) in group 4.

STOOLS

pH and Titration.—The stools from infants in group 1 had a mean pH of 5·2 for the first week of life (Table IV). They required a mean 0·06 ml acid for titration to pH 4·5. Stools from infants in group 2 had a mean pH of 5·9 with an average titratable alkalinity of 0·30 ml. Those from infants in group 3 had an average pH of 5·5 and titratable alkalinity of 0·18 ml up to day 7. Once bicarbonate was stopped pH increased to an average 6·2 for the remaining three days. The amount of acid needed for titration rose to a mean 0·46 ml. Infants in group 4 showed a similar pattern. The mean stool pH up to day 7 was 5·4 with a titratable alkalinity of 0·12 ml. After the removal of trometamol from the milk pH increased to a mean 6·2 and titratable alkalinity to 0·56 ml.

TABLE IV—*Stool pH and Titratable Alkalinity (10N Lactic Acid) in the 40 Infants in the Study Groups. Mean ± S.D.*

Group	Days After Birth			
	Days 2–7		Days 8–10	
	pH	Acid (ml)	pH	Acid (ml)
1	5·2 ± 0·4	0·06 ± 0·03	—	—
2	5·9 ± 0·3	0·30 ± 0·17	6·0 ± 0·3	0·40 ± 0·24
3	5·5 ± 0·4	0·18 ± 0·20	6·2 ± 0·4	0·46 ± 0·20
4	5·4 ± 0·4	0·12 ± 0·17	6·2 ± 0·4	0·56 ± 0·40
	Group 2 differs from others, $P < 0{\cdot}0005$.		Groups 2, 3, and 4, $P > 0{\cdot}1$.	

Cultures.—Stools from infants in group 1 had an average *E. coli* count of 2×10^8 and a lactobacilli count of 2×10^{10} organisms per gramme for the first week (Table V). Stools from infants in group 2 showed a reversal of this pattern. *E. coli* counts averaged $5{\cdot}4 \times 10^{10}$ and lactobacilli counts $5{\cdot}4 \times 10^9$. The addition of alkali to the milk promoted colonization of the bowel with organisms similar to those in breast milk stools. In group 3 the mean *E. coli* count for the first week was $7{\cdot}2 \times 10^9$. This rapidly increased to a mean of $4{\cdot}8 \times 10^{11}$ over the next three days, after the removal of bicarbonate. Mean lactobacilli counts before and after the removal of bicarbonate were $6{\cdot}4 \times 10^{11}$ and $5{\cdot}3 \times 10^9$ respectively. Stools from infants in group 4 averaged *E. coli* counts of $3{\cdot}2 \times 10^8$ and lactobacilli counts of $1{\cdot}3 \times 10^{10}$/g for the first seven days. Once trometamol had been removed these counts were reversed. *E. coli* counts now averaged $6{\cdot}4 \times 10^{10}$/g, while the lactobacilli count was a mean 2×10^8.

Discussion

Titration of cow's milk with alkali to a pH of 7·20-7·40 has several striking consequences. The altered milk has a bacteriostatic effect on specific *E. coli* in vitro; it promotes early growth

TABLE V—*Stool* E. coli *and Lactobacilli Counts and Range in the 40 Infants in the Study Groups*

Group	Days After Birth			
	Days 2–7		Days 8–10	
	E. coli/g	Lactobacilli/g	*E. coli*/g	Lactobacilli/g
1	2×10^8	2×10^{10}	—	—
Range	5×10^5–5×10^8	4×10^8–4×10^{10}	—	—
2	$5{\cdot}4 \times 10^{10}$	$5{\cdot}4 \times 10^9$	$4{\cdot}5 \times 10^{11}$	$2{\cdot}4 \times 10^9$
Range	5×10^4–5×10^{11}	5×10^5–1×10^{11}	8×10^8–5×10^{13}	5×10^8–1×10^{10}
3	$7{\cdot}2 \times 10^9$	$6{\cdot}4 \times 10^{11}$	$4{\cdot}8 \times 10^{11}$	$5{\cdot}3 \times 10^9$
Range	5×10^5–1×10^{11}	4×10^8–5×10^{12}	2×10^{11}–1×10^{11}	2×10^8–4×10^{10}
4	$3{\cdot}2 \times 10^8$	$1{\cdot}3 \times 10^{10}$	$6{\cdot}4 \times 10^{10}$	2×10^8
Range	5×10^5–1×10^{11}	5×10^7–3×10^{10}	2×10^9–3×10^{11}	1×10^6–1×10^{10}
	Group 2 differs from others, $P < 0{\cdot}0005$.		Groups 2, 3, and 4, $P > 0{\cdot}1$.	

in the newborn baby, and alters the pH and microflora of the infant's stool. The changes appear to simulate those produced by human milk.

Human milk and bovine colostrum are known to have a bacteriostatic effect on specific *E. coli* in the pH range of 7·20-7·33 (Bullen *et al.*, 1972). A similar effect was observed in reconstituted cow's milk (pH 7·40), but the reason for this inhibition is unknown. Bullen *et al.* (1972) indicated that the bacteriostatic effect could be enhanced by iron-binding proteins provided the pH is maintained above 7·0. They thought that the alkaline condition of the small bowel would be suitable for the inhibition of *E. coli*, particularly if milk reached this region in an undigested state. In support of this theory they noted the works of Mason (1962), who showed that stomach pH does not fall to a stage at which casein hydrolysis can occur, and Henderson (1942), who pointed out that milk passes rapidly into the newborn's duodenum during feeding. It is likely that in these circumstances the bacteriostatic effect of titrated cow's milk shown in vitro may continue to function in the small bowel.

The bacteriostatic property of titrated milk may be of practical significance in infant feeding. Milk formulas easily become contaminated with bacteria when terminal sterilization is not practised. This hazard is most pronounced in the homes of low socioeconomic groups, where up to 50% of feeds show contamination with enteric organisms. When such feeds are prepared in bulk and stored for more than 24 hours contamination by *E. coli* can be as high as 38% (Kendall *et al.*, 1971).

The stool flora of infants on titrated cow's milk resembled that of breast-fed babies. Bullen and Willis (1971) indicated that while *E. coli* counts were low in breast-fed infants and lactobacilli counts were high, the situation was reversed in bottle-fed babies. In the present study *E. coli* counts remained below 10^{10} per gramme while infants were fed on titrated milk. A significant increase of *E. coli* organisms was noted once alkali had been removed from the feeds. *E. coli* counts in the stools now approached those of infants on unaltered cow's milk. Stool lactobacilli counts were inversely related to the *E. coli* counts.

Stools of infants on alkali resembled those of breast-fed babies in that they were equally acid and had a reduced buffering capacity. Ross and Dawes (1954) thought that the preponderance of lactobacilli in the bowels of breast-fed babies and the relatively low pH were major factors responsible for resistance to gastroenteritis. It is likely that titrated cow's milk leading to a stool with a high lactobacillus count and low pH could provide similar resistance for the bottle-fed baby.

The growth promoting effect of titrated cow's milk was similar to that of breast milk. Consistent early weight gain of infants on sodium bicarbonate could not be attributed to fluid retention or to an increased intake of calories. Serum sodium levels were normal. The humanized cow's milk had a sodium content of 11 mEq/l., and the maximum daily intake of sodium by infants in the trial was estimated at 12 mEq. No recommendation can be given for the addition of sodium bicarbonate to milks containing a higher content of sodium. This compares favourably with the calculated maximum amount of 13 mEq a day for infants on unaltered cow's milk. Weight gain was not specific for sodium bicarbonate as infants receiving the electrolyte-free trometamol solution showed a similar weight and stool pattern.

A major point of consideration in the weight gain concerns the effect of bowel flora on the host. In this study a striking relation was noted between the numbers of *E. coli* in stool cultures and the growth pattern. During the stages of weight lag large numbers of *E. coli* were present in the stools. Intestinal flora are known to affect growth in health and disease. They play a major part in conditions such as the blind loop syndrome (Brown, 1969), megaloblastic anaemia (Foy *et al.*, 1951), and kwashiorkor (Smythe, 1958). In healthy infants (Robinson, 1952) and in animals growth promotion has been achieved by an alteration of the bowel flora with antibiotics. Piglets fed a diet containing sulphonamides showed improved daily weight gain. Their stools had a reduced *E. coli* count during the period of weight gain whereas lactobacilli remained unaffected (Wahlstrom *et al.* 1950). It has been postulated that the increase in growth produced by antibiotics could be due to the destruction of bacteria which compete with the host for foodstuffs, to an increase in growth factors produced by bacteria, or to the elimination of toxic compounds released by bacteria (Stokstad, 1954). Titrated milk may act in a similar way by preventing the overgrowth of *E. coli* organisms which could compete with the host for nutrition.

The growth lag noted in infants on unaltered cow's milk could not be attributed to an associated metabolic acidosis. To a certain extent the pattern resembled that described by Kilderberg (1964) in premature infants. He specifically noted that weight lag preceded metabolic acidosis, and it is feasible that the acidosis was due to the excessive acid load in artificial cow's milk. The growth achieved by his group of immature infants on sodium bicarbonate may have been due to an alteration of bowel flora rather than to the correction of metabolic acidosis per se.

Milk which has been titrated with alkali appears to be a more physiological food for the newborn infant than unaltered cow's milk.

This study was confined to correcting milk acidity by the addition of sodium bicarbonate or trometamol. The alkali was given for a short period only, and may be contraindicated for long-term use. The question whether it is more feasible to remove the acid radicals from cow's milk or to titrate the milk with alkali has not been examined.

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