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The Effect of Thiocyanate in Nutrition on the Iodine Content of Cow's Milk

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With 1 figure in 3 details and 5 tables

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When the claim (1) that milk produced on *Cruciferae* plants has an antithyroid effect has been investigated in our institute in recent years, special attention has been paid to goitrogenic substances in these plants and in milk (2). GMELIN and VIRTANEN (3) showed that the thiocyanate ion (SCN^-) which causes „cabbage goitre“ is formed in crushed *Brassica* plants enzymically from the precursors glucobrassicin (4) and its N_1 -methoxy derivative, neogluco-brassicin (5). These precursors were found in different *Brassica* species and their structures were elucidated. The thiocyanate formed when the cow is fed on *Brassica* plants is secreted partly into the milk, but its concentration in milk does not rise essentially above 1 mg% even when very large doses of thiocyanate are used (6). VILKKI et al. (7) have shown that this level does not yet cause any disturbances in the uptake of iodine by the thyroid gland of man. In feeding

experiments of long duration it has not caused goitre in rats, either (2). This is true provided that the lack of iodine in the nutrition is not so great that even a small disturbance in iodine uptake would lead to the development of goitre.

VIRTANEN, KREULA, and KIESVAARA (8) found that also the strong antithyroid compound, L-5-vinyl-2-thiooxazolidone (goitrin), - which is formed in many *Brassica* fodder plants when these are crushed (9) - is transferred to milk from the rumen of the cow in so minute amounts that it is of no practical importance.

Even though the amount of thiocyanate which is formed from a large amount of cabbage or rutabaga does not make milk goitrogenic, it may have an indirect unfavourable effect on the iodine metabolism of the animal. In addition to a disturbed iodine uptake by the thyroid gland, it has been observed that the iodine content in milk produced on different *Brassica* species has decreased while the thiocyanate content has increased. On the other hand, surprisingly high iodine contents have been observed in milk after the feeding of thiocyanate (10) has been discontinued. These observations led to the question how thiocyanate affects the iodine content of cow's milk. In order to elucidate this question, three cows were fed 1.5 g of SCN⁻ as the potassium salt daily during one week and 3.0 g daily during the next week. These amounts of thiocyanate correspond to the amounts which cows take in when *Brassica* fodder plants are fed in abundance. The milk samples were analysed for both iodine and thiocyanate.

Experimental

Methods

A modification of the method of BARKER et al. (11) was used with slight alterations for the determination of the total iodine in milk and fodder. Using this modification, VILKKI (12) had earlier determined the iodine contents of the most common foodstuffs in Finland, especially milk. 0.25-1.0 ml of milk or 50-300 mg of ground fodder, 1 ml of 10% zinc sulphate and 1 ml of 5 N potassium hydroxide were measured into glass tubes. The mixture was dried for 18 hours at 110 °C and then for 2 hours at 150 °C, after which it was heated at 600 ° for 2 hours. The ash was dissolved in 5 ml of 0.08 N potassium arsenite. 3 ml of the clear centrifuged liquid was acidified with 1 ml of 7 N sulphuric acid which contained 10% (w/v) sodium chloride and warmed to 37 °. 1 ml of 0.01 N cerium ammonium sulphate was added and the optical density of the solution was measured against water with a Klett-Summerson colorimeter (filter 42) 6 and 12 minutes after the addition. The amount of iodide catalysing the reduction of Ce (IV) was obtained by comparing the difference of the corresponding transmission values with those of the samples after the addition of 0.04 µg of iodide.

The determination of the thiocyanate in the milk was performed according to BARKER's (13) method. The protein in 10 ml of milk was precipitated with 10 ml of 20% trichloroacetic acid, 1 ml of 5% (w/v) iron (III) nitrate in 2.5% (v/v) nitric acid was added to 5 ml of the filtrate, and the colour was measured with the KLETT-SUMMERSON colorimeter (filter 50). A solution containing 5 ml of the filtrate and 1 ml of water was used as a blank. The thiocyanate content of the sample was obtained by comparing the results with a standard curve.

The arrangement of the feeding experiment

The feeding experiment was carried out with three Ayrshire cows with a fairly equal milk production on the Joensuu farm during the indoor feeding period 7.2.-12.3.1962.

Aino,	5 years old, calved on January	14, 1962
Ella II,	3 " " " September	25, 1961
Elo II,	4 " " " August	31, 1961

The average rations and the average iodine contents of the different feeds given during the experiment are shown in Table 1.

Table 1.

The rations and iodine contents of the different feed components used in the experiment

Feed ration/day	Iodine, $\mu\text{g}/\text{kg}$ fodder	Iodine, mg, in daily ration
25 kg maize-AIV-silage	190	4.8
5 „ crushed oats	45	0.2
3 „ hay	115	0.3
3 „ straw	350	1.1
40 l water	15	0.6
	Total	7.0 mg

The feeding experiment was continued during five periods of one week. Additional portions of potassium iodide and thiocyanate, mixed with a small amount of crushed oats, were given daily at 2-2.30 p. m. to each cow as shown in Table 2.

Table 2. Intake of additional KI and KSCN in different test periods

Week	Days	KI mg	KSCN g	Cows
1st	1st-5th	0	0	all three
2nd	6th-12th	13.1*	0	„
3rd	13th-19th	13.1	2.5**	„
4th	20th-26th	13.1	5.0***	„
5th	27th-33rd	13.1	0	Elo II
		0	0	Aino and Ella II

* 10 mg I⁻

** 1.5 g SCN⁻

*** 3.0 g SCN⁻

Table 3. The average milk production in different test periods

Cow	Week	Morning milk kg/day	Evening milk kg/day	Production kg/day
Aino	1st	8.1	7.1	15.3
	2nd	7.7	6.5	14.1
	3rd	7.7	6.5	14.1
	4th	7.0	6.1	13.1
	5th	7.3	6.2	13.5
Ella II	1st	7.4	6.3	13.7
	2nd	7.1	5.3	12.4
	3rd	6.2	4.7	10.9
	4th	6.2	5.2	11.5
	5th	5.6	4.7	10.3
Elo II	1st	7.6	6.2	13.8
	2nd	7.2	5.7	12.9
	3rd	6.4	5.1	11.5
	4th	5.7	4.8	10.6
	5th	5.8	4.7	10.5

The cows were milked in the morning at 6-6.30 and in the evening at 5-5.30. Determinations of iodine and thiocyanate were carried out on each milk sample. The average milk production of the cows during the different periods is shown in Table 3.

Results

The results presented in Fig. 1 and Tables 4 and 5 show that the continuous feeding of thiocyanate effectively decreased the iodine content of the milk of each cow although the contents revealed individual differences.

The thiocyanate (SCN^-) contents of the milk during the experiment are seen in Table 4.

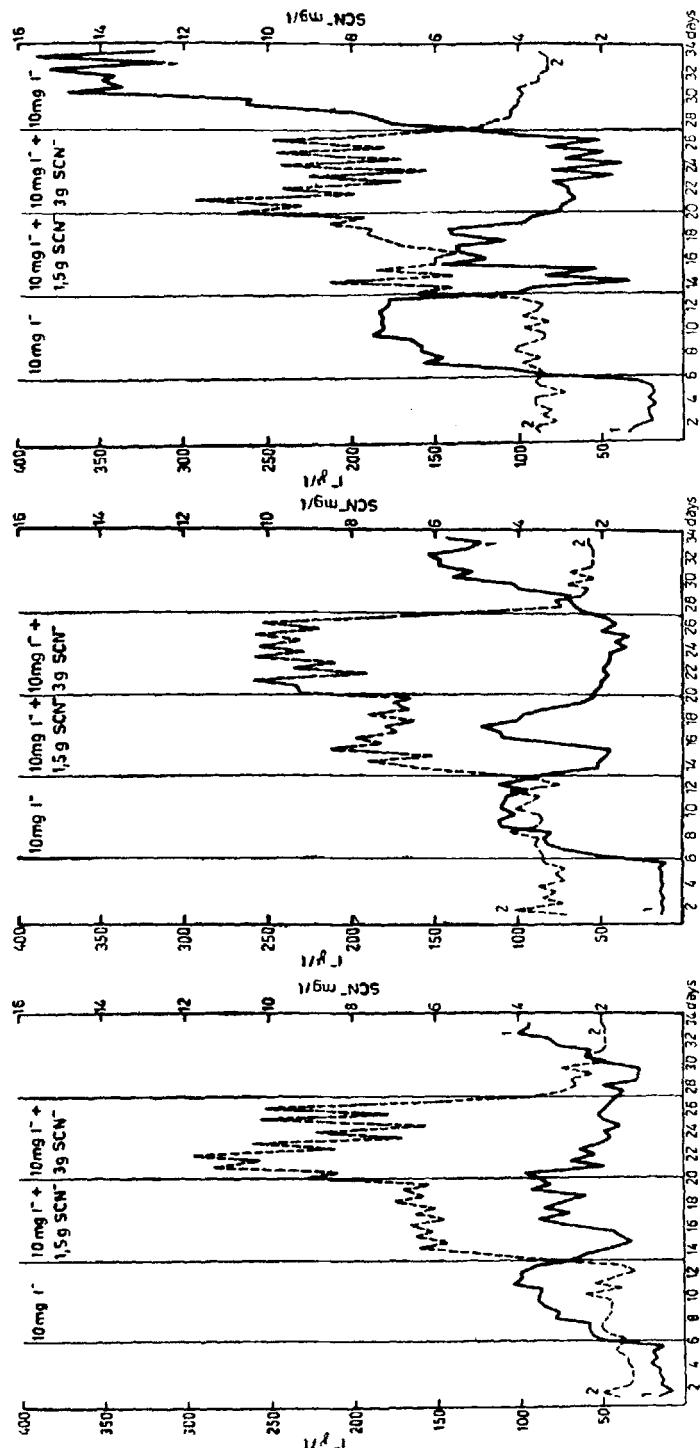
Table 4. Secretion of thiocyanate into milk

Cow	The average SCN^- content of milk, mg/l				Secretion of SCN^- into milk during the test, % of given amount
	Before SCN^- feeding 1st-12th days	1.5 g SCN^- /day 14th-19th days	3.0 g SCN^- /day 23rd-26th days	After SCN^- feeding 31st-33rd days	
Aino	1.7	6.2	8.1	1.9	3.6
Ella II	3.4	6.8	9.3	2.2	2.2
Elo II	3.5	6.3	8.1	3.4	2.0

A daily ration of 1.5 g of SCN^- per cow raised the thiocyanate concentration in the milk in 24 hours to 0.6-0.7 mg % independently of the initial level. With a double ration, the thiocyanate content of Ella II's milk rose to 0.9 mg %, and that of Aino's and Elo II's milk temporarily to 1.2 mg %, from which level it soon decreased to an average level of 0.8 mg %. During the whole experiment the total secretion in Aino's milk was almost twice (3.6% of the given amount of SCN^-) that in the other cows (2.0-2.2% of the given amount). The thiocyanate concentration, which was usually higher in the evening milk than in the more abundant morning milk, decreased rapidly to the initial level after the feeding of thiocyanate was discontinued. The decrease was somewhat more rapid than in the previous experiments of VIRTANEN and GMELIN (6) in which no additional iodide was given.

The changes that occurred simultaneously in the iodine content of the milk are seen in Fig. 1.

During the first week, when the cows received 7 mg of iodine per day in the fodder, the iodine content of the milk amounted to about 10-20 μ g per l, which corresponded to 2.6-4.1% of the daily iodine intake. The daily addition of 10 mg of I^- in the second week increased the iodine content of the milk to 90-175 μ g per l, which corresponded to 7.4-13.2% of the total iodine fed. In the third week, the iodine content of the milk decreased abruptly already after the first dose of thiocyanate (1.5 g of SCN^- per 24 hours) and fell to a minimum on the second or third day (3.1-4.4% of the total iodine secreted into the milk), rose then almost to the maximum of the previous iodide period, but began to decrease again in two test cows after 3-4 days. A double dose of thiocyanate (3 g of SCN^-) caused the iodine content to decrease to a relatively stable level which corresponded to the minimum level obtained already with the first dose (1.5 g) or to about $1/3$ of the concentration reached before the thiocyanate period (3.0-4.3% of the total iodine secreted into the milk). The average amounts of iodine secreted into milk during the experimental periods are shown in Table 5.



Aino

Elo III

Fig. 1. The effect of a continuous feeding of iodide and thiocyanate on the iodine (1) and thiocyanate (2) content of the milk. The amounts of the daily doses of iodide and thiocyanate (SCN^-) are given in the upper edge of the Figure in connection with each test period.

Elo II

The discontinuation of the thiocyanate feeding caused a remarkable change. Although iodide was no longer given to two cows, the iodine content of the milk rose so high that it either reached or exceeded considerably the high iodine level that prevailed before the feeding of thiocyanate when the cows received 10 mg of I^- per day. It was 5–11 times the iodine level of the milk in the initial period of the experiment before the feeding of iodide (Table 5 and Fig. 1, Aino and Ella II). The feeding of iodide was continued to one of the cows (Elo II) during the last period when thiocyanate was no longer given; the iodine content of the milk increased to twice the content in the second week of the experiment when the cow received the same quantity of iodide but no thiocyanate (Fig. 1, Elo II). Irrespective of whether the cow took in only the 7 mg of iodine present in the fodder, or an additional 10 mg of I^- , on the average 20% of iodine fed was secreted into the milk on the sixth day after the thiocyanate feeding had been discontinued. The average secretion of iodine 4–7 days after the thiocyanate feeding ended varied from 15 to 21%.

Discussion

All the feeding experiments (those reported in this paper and four others not described) have shown that the feeding of thiocyanate to cows strongly decreases the iodine content of the milk. The results suggest an active mechanism which concentrates iodine in the mammary gland of the cow. BROWN-GRANT (14) and POTTER et al. (15) have established this kind of mechanism in gnawers and its prevention with thiocyanate in injection experiments with ^{131}I and SCN^- . GARNER et al. (16) observed in their feeding experiments with cows that the secretion of radioiodine into milk was prevented by 10 g of sodium thiocyanate which according to our observations greatly exceeds the amount of thiocyanate which the cow can get daily even

Table 5.
Daily doses of I^- and SCN^- (lower part of the table), and the average secretion of iodine into milk (upper part of the table)

Cow	1st week				2nd week				3rd week				4th week				5th week			
	2nd–5th days		% of dose		8th–12th days		% of dose		16th–19th days		% of dose		22nd–26th days		% of dose		28th–33rd days		% of dose	
	% of dose	$\mu g/l$	% of dose	$\mu g/l$	% of dose	$\mu g/l$	% of dose	$\mu g/l$	% of dose	$\mu g/l$	% of dose	% of dose	$\mu g/l$	% of dose	% of dose	$\mu g/l$	% of dose	% of dose	$\mu g/l$	
Aino	3.5	16	7.7	90	5.7	70	3.7	49	15.0	7.6	44	19.6	13.9	21.1	34.7					
Ella II	2.6	13	7.4	103	5.5	86	3.0	44												
Elo II	4.1	21	13.2	175	7.6	113	4.3	68												
I^- (mg) given in 24 h		7.0				17.0					17.0					17.0			7.0 (17.0 Elo)	
SCN^- (mg) given in 24 h		0				0					1.5					3.0			0	

when it is fed large quantities of *Brassica* plants. In our experiments the effect has been achieved with quantities corresponding to those cattle receive normally. The mechanism of iodide uptake by the mammary gland is obviously very sensitive to the effect of thiocyanate, more sensitive than the mechanism of iodide uptake by the thyroid gland. Also the fact that REINEKE (17) recently succeeded in achieving a similar result with a goat in injection experiments, using a quantity of thiocyanate which did not yet inhibit iodide uptake by the thyroid gland, supports this view.

Because thiocyanate prevents the secretion of iodine into milk, it protects cows from getting goitre when the iodine content of their feed is low. On the other hand, the low iodine content of milk is harmful from the point of view of human nutrition.

The finding that when the feeding of thiocyanate was discontinued, the iodine content of milk increased within a few days to a value 2 to 11 times as high as that attained with the corresponding iodine doses before thiocyanate was fed is especially striking. It seems at first sight as if iodine had accumulated somewhere else in the organism during the feeding of thiocyanate and was secreted into the milk after the influence of thiocyanate had ceased. However, it must be taken into consideration that even in the last experimental period the amount of iodine secreted into milk was only about 20% of the quantity given. A hypothesis of an accumulation of iodine in the organism during the thiocyanate feeding is thus not necessary to explain the enormous rise in the iodine level of the milk when thiocyanate was omitted. It is more probable that the feeding of thiocyanate activates a mechanism in the organism which stimulates the iodide uptake by the mammary gland. As a result of this activation, the secretion of iodine into milk rises when thiocyanate is no longer given. The fluctuations in the iodine content of the milk when a lower dose of thiocyanate (1.5 g of SCN⁻/cow) was given during the third feeding period also support this assumption. A sharp decrease in the iodine content was followed by a strong increase and then a decrease. Only when 3.0 g of thiocyanate was fed daily to a cow, did the low level remain constant.

As we have found so far, the iodide uptake by the mammary gland is very similar to that of the thyroid gland. When thiocyanate inhibits the iodide uptake by the mammary gland, the capacity of the gland to take up iodide is stimulated. This state of stimulation continues even after the feeding of thiocyanate is stopped, and it is the cause for the exceptionally high iodine content of the milk when thiocyanate is removed from the food. It is possible that the results presented in this paper are the first hints of an analogy between the mammary gland and the thyroid gland regarding the uptake of iodide.

According to previous investigations in this laboratory, the continuously increasing use of *Cruciferae* plants in the feeding of cows, especially in the northern areas, does not make the milk goitrogenic. The results presented above reveal an indirect danger: the feeding of *Cruciferae* plants decreases the iodine content of milk greatly. This effect must be taken into consideration particularly in a country like Finland where milk is the most important source of iodine in food (12). For this reason the general use of iodized salt in human nutrition should be emphasized more than ever before. The use of iodine-containing salt mixtures in cattle feeding in order to eliminate possible disadvantages caused by thiocyanate in the fodder is also recommended.

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Summary

When cows are continuously fed amounts of potassium thiocyanate corresponding to the quantity formed from 15-30 kg of the marrow kale or rutabaga per day (1.5 and 3.0 g of SCN⁻, respectively), the thiocyanate concentration in the milk rises and the iodine content of the milk decreases. When the feeding of thiocyanate is discontinued, the iodine content of the milk rises much above the initial level during a few days. The results suggest that this is due to a mechanism that regulates the uptake of iodide by the mammary gland.

Zusammenfassung

Wenn die Kühe kontinuierlich mit solchen Mengen von Kaliumrhodanid gefüttert werden (resp. 1,5 und 3,0 g SCN⁻), welche der aus 15-30 kg Markstammkohl oder Kohlrübe pro Tag entstehenden Quantität entsprechen, steigt die Konzentration von Rhodanid in der Milch unter gleichzeitiger Senkung des Jodgehalts. Wenn die Fütterung von Rhodanid nicht mehr fortgesetzt wird, steigt der Jodgehalt stark über das Anfangsniveau während ein paar Tagen. Die Resultate weisen darauf hin, daß dies auf einen Mechanismus zurückzuführen sei, der die Jodidaufnahme der Milchdrüse regelt.

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