

## On the Transfer of Thiocyanate from Fodder to Milk

ARTTURI I. VIRTANEN and ROLF GMELIN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

In connection with our studies on goitrogenic factors in crucifers, especially those belonging to the *Brassica* species and varieties, the enzymatic formation of the thiocyanate ion  $\text{SCN}^-$  from some mustard oil glucosides was found<sup>1</sup>. It has been known that  $\text{SCN}^-$  inhibits iodine uptake by the thyroid gland, and there is reason to assume that  $\text{SCN}^-$  is one member of the *Brassica* factor which causes goitre in experimental animals.

An important question is to what extent the  $\text{SCN}^-$  which is formed when the cows chew the fodder is transferred from the rumen to the milk. In order to elucidate this problem we have performed feeding experiments with cows by giving them different amounts of potassium- and ammonium thiocyanate mixtures.

According to our determinations about 0.2 mg % of  $\text{SCN}^-$  is generally found in the mixed milk during the indoor freeing from the big dairies in Southern Finland. In this area stable feeding commonly consists of hay (mostly timothy, often mixed with red clover), oats, silage (AIV) made of red clover-grass mixtures or sugar beet tops, and sugar beet pulp. If beets are used in the feeding, they are mainly mangels or fodder sugar beets. Marrow kale is still grown to a comparatively small extent. The growing of swedes is also slight.

If the daily feeding of the cows contains about 30 kg of marrow kale or the same amount of swedes, the  $\text{SCN}^-$  content of the milk is about 0.5 to 0.8 mg % according to our determinations. As already mentioned, the feeding of marrow kale is not general in Finland, and on the farms which grow it, it is restricted to October–November. Swedes again may be fed all the winter, but the daily rations are small on those relatively few farms on which they are grown. It is probable that the growing of marrow kale will increase in Finland. In Northern Finland, up to the polar circle and even beyond it, winter rape and turnip rape will probably become important raw

material for silage on the basis of experiments and the experience obtained during the last years. The thiocyanate content of the milk will thus increase in this large area.

### *Determination of $\text{SCN}^-$ in milk and in urine.*

A modification of Barker's method<sup>2</sup>, essentially as described by Michajlovskij and Langer<sup>3</sup>, was used for the determination: 10 ml of 20 % trichloroacetic acid was added to 10 ml of milk. 5 ml of the clear filtrate was treated with  $\text{Fe}^{3+}$ -reagent. The red brown coloration was measured in a Klett-Summerson photometer with filter 50 and compared with a standard  $\text{SCN}^-$  curve. 5 ml of milk filtrate + 1 ml of water, and 1 ml of  $\text{Fe}$ -reagent + 5 ml of water was used for background correction.

In the case of urine, exact determinations of  $\text{SCN}^-$  values were not possible since phenolic substances disturb the Barker method as well as the Aldridge method. The excretion of  $\text{SCN}^-$  was followed by subtraction of the colorimetric values in parallel experiments, *i. e.* in urines of cows fed with  $\text{SCN}^-$ , and cows which had received identical feeding but without addition of  $\text{SCN}^-$ . The found values can therefore be regarded only as approximative values.

*First feeding experiment.* On January 16, 1960, a mixture of 2 g of  $\text{NH}_4\text{SCN}$  and 2 g of  $\text{KSCN}$  was fed to the Ayrshire cow Häyri (calved on March 17, 1959) at 7.20 and 17.20; total amount of  $\text{SCN}^-$ , 5.46 g. The first milk sample was taken just before the feeding of thiocyanate. 0.24 mg % of  $\text{SCN}^-$  was found in it. The milking times were regularly at 6.30 and 17.10, and a milk sample was taken each time for the determination of  $\text{SCN}^-$ . Twelve days after the feeding of thiocyanate, a milk sample was taken only once a day for analysis, and after fifteen days only now and then. During the whole test period, the milk production of the cow was mostly 6.5 to 7.5 kg in the morning and 5.0 to 6.0 kg in the evening. The daily production ranged from 11 to 13 kg of milk with 3.5 % of fat. The  $\text{SCN}^-$  content of the milk is shown in Fig. 1.

The results show that 10 h after the first portion of thiocyanate, the  $\text{SCN}^-$  content of the milk has risen from the initial value of 0.24 mg % to 0.9 mg %. The feeding of the second portion in the same evening raised the  $\text{SCN}^-$  content of the following morning milk to 1.4 mg %, after which the content decreased in 24 h to about 0.7 mg %, where it remained for over ten days. The original  $\text{SCN}^-$  content of the milk was reached only after about four weeks after the feeding of thiocyanate. The total milk

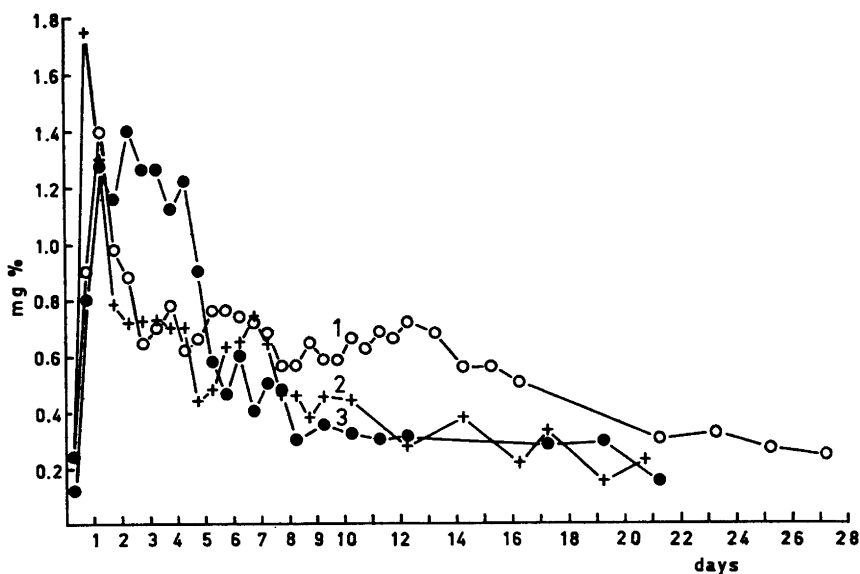


Fig. 1.  $\text{SCN}^-$  content of milk from cows fed different doses of thiocyanate. 1, Cow Hairy, fed 5.46 g of  $\text{SCN}^-$  in two doses on January 16, 1960; 2, Cow Hairy, fed 6 g of  $\text{SCN}^-$  in one dose on February 29, 1960; 3, Cow Mila, fed 24 g of  $\text{SCN}^-$  during four days in eight doses.

yield was about 315 kg during this time. About 1 000 mg or some 18 % of the 5.46 g of  $\text{SCN}^-$  fed was excreted in the milk. No  $\text{SCN}^-$  determinations were performed on the urine.

*Second feeding experiment.* A mixture of 1.95 g of  $\text{NH}_4\text{SCN}$  and 2.5 g of  $\text{KSCN}$ , corresponding to 3.0 g of  $\text{SCN}^-$ , was fed twice a day at 7.00 and 17.00, in total  $8 \times 3 \text{ g} = 24 \text{ g}$  of  $\text{SCN}^-$ , to the Ayrshire cow Mila (calved on September 26, 1958). The first milk sample was taken just before the feeding of thiocyanate was begun. 0.12 mg % of  $\text{SCN}^-$  was then found in the milk, *i. e.* half of Hairy's initial value, although the feeding of both cows was the same: 4 kg of oats, 4 kg of hay, 20 kg of AIV silage made of a red clover-grass mixture, and 2 kg of oat straw. The  $\text{SCN}^-$  content of the milk yields are shown in Fig. 1.

The  $\text{SCN}^-$  content of Mila's milk also rose to 1.4 mg %, remaining at about this level during the four days in which the thiocyanate preparation was fed. A decreasing tendency appeared, however, already at the fourth day of the feeding. In spite of the fact that Mila got altogether well four times more  $\text{SCN}^-$  than Hairy, the  $\text{SCN}^-$  content of Mila's milk decreased more

rapidly, reaching 0.3 mg % already after eight days from the beginning of the feeding experiment. Obviously  $\text{SCN}^-$  was more rapidly oxidized in Mila's organism than in Hairy's. The extremely low initial value of 0.12 mg % of  $\text{SCN}^-$  in Mila's milk was possibly due to this fact. The daily milk production ranged from 10 to 11.5 kg with 4.7 % of fat. About 900 mg of  $\text{SCN}^-$ , or less than 4 %, of the 24 g of  $\text{SCN}^-$  fed was excreted in the milk. The total milk yield was about 220 kg during the three weeks in which the  $\text{SCN}^-$  content exceeded the initial value. Also in this case no  $\text{SCN}^-$  determinations were performed on the urine.

The amounts of thiocyanate fed to the cows in the experiments related above were so high that cattle can hardly get larger or even as large ones when plenty of crucifers are used in the feeding. We have found that about 10 mg % of  $\text{SCN}^-$  is formed in crushed marrow kale, and hence a daily dose of 6 g of  $\text{SCN}^-$  would theoretically presuppose a ration of 60 kg of marrow kale a day, which is hardly eaten by a cow. Daily rations of more than 30 kg a day are usually not recommended because of the danger of diarrhea.

Table 1. Excretion of  $\text{SCN}^-$  in urine. On February 2, at 7.00 the cow H yri was given 6 g of  $\text{SCN}^-$  (as  $\text{KSCN} + \text{NH}_4\text{SCN}$ ) in one dose.

date	time	amount of urine/l	$\text{SCN}^-$ mg/100 ml of urine	total $\text{SCN}^-$ in urine/mg
29.II	10.55	2.0	7.8 (-6)	156 (36)
29.II	15.50	1.5	23.2 (-6)	348 (258)
29.II	22.30	1.5	58.0 (-6)	870 (780)
29.II	23.55	2.2	12.2 (-6)	268 (136)
1.III	01.16	2.7	8.0 (-6)	216 (54)
1.III	02.56	3.0	9.2 (-6)	276 (96)
1.III	04.24	2.1	8.6 (-6)	181 (55)
1.III	05.45	0.8	13.8 (-6)	110 (62)
15.8 1/24 h				2 425 mg
				(1 477 mg $\text{SCN}^-$ /15.8 l of urine)

*Third feeding experiment.* The cow H yri, the test animal in the first experiment, was given 3.9 g of  $\text{NH}_4\text{SCN}$  and 5 g of  $\text{KSCN}$ , corresponding to 6 g of  $\text{SCN}^-$ , in one dose on February 29, 1960 at 7.00. In this experiment the  $\text{SCN}^-$  content of the urine was also determined during 24 h after the feeding of thiocyanate. The  $\text{SCN}^-$  content of the milk before the start of the experiment was 0.24 mg %, *i. e.* the same as at the beginning of the first experiment. Ten hours after the feeding of thiocyanate, the  $\text{SCN}^-$  content of the milk had risen to 1.75 mg %. The  $\text{SCN}^-$  content of the milk is shown in Fig. 1, and that of the urine in Table 1.

Before the feeding of thiocyanate, the  $\text{SCN}^-$  content of the cows' urine was on the average about 6 mg of  $\text{SCN}^-$  per 100 ml. When this amount was subtracted from the  $\text{SCN}^-$  found in H yri's urine after the feeding of thiocyanate, it was established that about 1.5 g of  $\text{SCN}^-$  was excreted in the urine during 24 h. The urine was not collected after this, but the  $\text{SCN}^-$  content of it had decreased so rapidly during the first day that an excretion of  $\text{SCN}^-$  in urine over 2 g, *i. e.* one third of the thiocyanate fed, is not probable. Since about 800 mg of the  $\text{SCN}^-$  fed was excreted in the milk, about half the amount fed has obviously been oxidized in the organism. This is of course only a rough estimation. In experiments with dogs Boxer and Rickards<sup>4</sup> found that about 30 % of  $\text{SCN}^-$  are metabolized in the body to  $\text{CO}_2$ .

On the basis of the above results, it does not seem probable that the  $\text{SCN}^-$  content

of cow's milk could rise much above 1 mg % in practice, even when large amounts of crucifers are included in the feeding of cattle. This is in agreement with the results of Wokes *et al.*<sup>5</sup> who found  $\text{SCN}^-$  values between 0.01 and 1 mg % in a large number of milk samples. The feeding of the cows was probably unknown to the authors (it is not mentioned in the paper), but obviously the samples analysed also included milk from herds fed with marrow kale which is a relatively common fodder plant on farms in England.

With normal feeding Wright<sup>6</sup> found 0.8 mg % of  $\text{SCN}^-$  and with kale feeding 4.6 mg % in goat's milk. Obviously goat's milk contains about four times more  $\text{SCN}^-$  than cow's milk, even if its chemical composition is very similar to that of cow's milk.

This investigation is a part of a research project under U. S. Public Law No. 480, 83rd Congress.

1. Gmelin, R. and Virtanen, A. I. *Acta Chem. Scand.* **14** (1960) 507.
2. Barker, H. J. *Am. Med. Assoc.* **106** (1936) 762.
3. Michajlovskij, N. and Langer, P. *Z. physiol. Chem. Hoppe-Seyler* **312** (1958) 26.
4. Boxer, G. E. and Rickards, J. C. *Arch. Biochem. Biophys.* **39** (1952) 7.
5. Wokes, F., Wedgwood, P. and Wyatt, J. *Biochem. J.* **50** (1952) xix.
6. Wright, E. *Nature* **181** (1958) 1602.

Received April 22, 1960.