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EXCRETION OF SELENIUM IN THE MILK OF SHEEP*)

By

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Selenium is excreted from the body through the mammary glands into the milk as well as by other routes. *Dudley & Byers* (1935) reported that up to 3.0 parts per million of selenium were present in the milk of cows that had been fed with seleniferous vegetation or had been given inorganic selenium compounds. *McConnell* (1948) demonstrated that selenium was transferred through the mammary glands of rats and that at least 2.5 to 9.3 per cent of injected selenium was present in young sucklings 24 hours after the last injection. A lactating mouse administered lucerne in which the proteins were labelled with radioactive selenium (Se^{75}) transferred 20 per cent of the given radioactivity to their young within 24 hours (*Jones & Godwin* 1963).

After intravenous injection of Se^{75} as selenious acid in an ewe most of the radioactivity excreted into the milk was found in the protein fraction (*Jones & Godwin* 1963). *McConnell & Roth* (1964) are of the opinion that in a bitch the administered inorganic Se^{75}-selenium was converted into organoselenium in the milk because nearly all the Se^{75} was present in proteins of the milk.

A number of studies have revealed that when selenium is given to pregnant ewes their lambs are protected against muscle degeneration. It also results in an increased growth of the lambs compared with that of the untreated controls (*Muth et al.* 1958; *McLean et al.* 1959; *Oldfield et al.* 1960). It has also been shown

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that lambs, which were born of ewes treated with selenium and themselves subsequently given this treatment when two or three weeks old, grew more rapidly than lambs treated in the same way but born of untreated ewes (*Hartley & Grant* 1961).

The selenium given to an ewe can be transmitted to the lamb via the placenta and via the milk. There are very few studies to elucidate the importance of these pathways. The transport of selenium to the lambs via the placenta has been studied by *Wright & Bell* (1964). They demonstrated a marked placental barrier for selenium. No data concerning the quantitative excretion of selenium compounds in the milk of ewes have been published.

The present investigation concerns the selenium excretion into the milk and its transfer into the lambs of ewes injected subcutaneously with organic or inorganic selenium compounds either before or after delivery.

MATERIAL AND METHODS

Se^{75}-sodium selenite was obtained in aqueous solution from The Radiochemical Centre, Amersham, England. At the beginning of the experiment the specific activity was 1.14 mC/mg. The solution was diluted with physiological saline to a concentration of 0.096 mg of selenium (corresponding to 0.11 mC Se^{75}) per ml.

Se^{75}-selenomethionine was obtained from The Radiochemical Centre, Amersham, England. The specific activity was 21.9 mC/mM. The substance was dissolved in physiological saline so that 1.0 ml contained 0.47 mg of selenomethionine (corresponding to 53 μC Se^{75}).

The animals were divided into 4 groups according to the form in which the selenium was administered and to the time when this occurred in relation to delivery. During the experimental period the animals received conventional fodder.

Group A 1. Ewe 244 was injected 29 days, and ewes 5, 106, and 261 12—13 days before delivery with 5.0 ml = 0.48 mg of selenium (0.54 mC Se^{75}) in the form of Se^{75}-sodium selenite. The injections were made subcutaneously over one shoulder-blade.

Group A 2. Ewes 8 and 103 were injected less than 5 hours after delivery and ewes 803 and 1, respectively, 24 and 48 hours after delivery with 5.0 ml = 0.48 mg of selenium (0.54 mC Se^{75}) in the form of Se^{75}-sodium selenite.

Group B 1. Ewes 273 and 275 were injected subcutaneously one month after delivery with, respectively, 1.0 ml = 0.096 mg of selenium (34 μC Se^{75}) and 0.85 ml = 0.082 mg of selenium (28 μC Se^{75}) in the form of Se^{75}-sodium selenite.

Group B 2. Ewes 274 and 278 were injected subcutaneously one month after delivery with 1.0 ml = 0.47 mg (53 μC Se^{75}) of Se^{75}-selenomethionine.

The ewes were of Swedish "lantras" type weighing between 38 and 50 kilos and varying in age from two to six years. In addition to the ewes, each group contained also the lambs of the respective ewes. The lambs were designated by the ewes' numbers, the index for single or twin lambs, and a sign indicating their sex, e.g. 5_1♀, 8_2♂ and 8_2♀.

Milk samples were taken between two hours and 15 days after delivery or administration of selenium. The time when the samples were taken is shown in Figures 1 and 4. The content of Se^{75} was determined in 1 ml of whole milk from each sample and in various milk fractions from ewes 106, 8, 273, 275, 274 and 278.

Blood samples from the ewes and the lambs were taken from the jugular vein.

The lambs 5_1♀ and 106_2♂ were anesthetized with barbiturates and bled to death immediately after birth. Lamb 8_2♂ was killed in the same manner 13 days after birth. Tissue samples (approximately 1 g) were taken for radioactive measurements. The weight of kidneys, liver, spleen, lungs and heart was determined.

The colostrum and the milk were fractioned according to *Smith* (1946, 1948). The cream was removed by centrifugation.

The casein (Fraction A) was precipitated by adding 0.1 N HCl to pH 4.5 and removed by centrifugation. The casein was dissolved in water after addition of 0.1 N NaOH to pH 6.5 and reprecipated twice. The filtrate after the casein precipitation was brought to pH 6 with 0.1 N NaOH and successive fractions were removed at 30 % (Fraction B), 50 % (Fraction C) and 90 % (Fraction D) saturation with ammonium sulphate.

The fractions were dissolved in water and reprecipitated at the same salt concentration and then dialyzed in water during 18 hours. The properties of the fractions B, C and D were investigated by means of paper electrophoresis. The electrophoresis was performed mainly according to *Nilsson* (1958), but high resolution buffer (*Aronsson & Grönwall* 1957) was used instead of veronal buffer. Before electrophoresis the samples were

dialyzed against buffer solution for 18 hours. The samples were then concentrated by dialysis against polyethylene glycol (*Kohn* 1959).

Fraction B: This fraction was electrophoretically very unitary and consisted of slow moving globulins. According to *Jenness et al.* (1956) these globulins are immunoglobulins and they will in this work be referred to as immune lactoglobulin I.

Fraction C: The main part of the fraction has electrophoretically the same properties as the previous fraction. The whole fraction will be referred to as immune lactoglobulin II.

Fraction D: This fraction, which corresponds to β-lactoglobulin in cow-milk, was very small in ewes. According to *McDougall* (1965) ewes seem to lack β-lactoglobulins and the small amounts found in this fraction may therefore be some protein not precipitated with lower concentration of ammonium sulphate. The fraction will be referred to as lactoglobulin.

The protein content in the milk and its different fractions were determined according to *Lowry et al.* (1951).

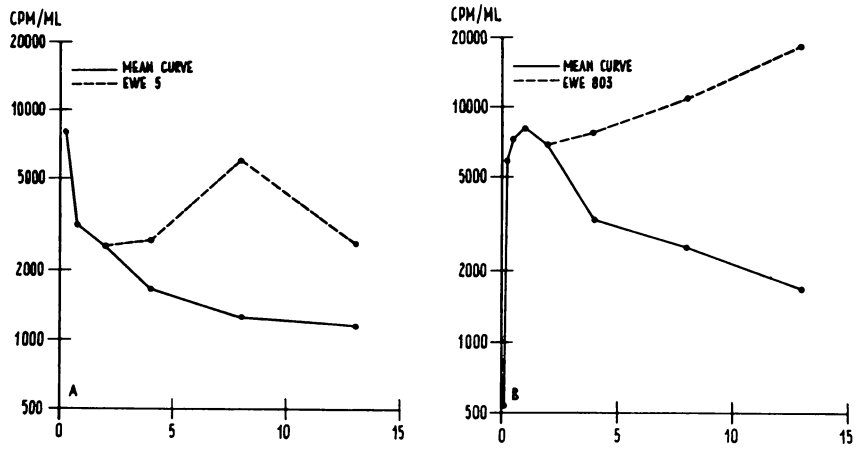
The radioactivity was measured with a well-type scintillation detector connected to a single-channel analyzer and a scaler.

RESULTS

The Se^{75}-concentrations in the milk of ewes injected with Se^{75}-sodium selenite before delivery (A 1) and immediately after delivery (A 2) are shown in Fig. 1 a and b. For the ewes in group A 1 the highest Se^{75}-concentration was obtained in the first milk samples. Thereafter it rapidly decreased, and after four days became relatively constant. Ewe 5, which was not suckled, had 48 hours after delivery a higher Se^{75}-content than the other ewes.

Already two hours after injection, Se^{75} was present in the milk of ewes in group A 2. The mean value curve for the Se^{75}-concentration in the whole milk in A 2 approached its maximum after 24 hours. Its value was then of the same order of magnitude as the highest mean value in A 1. Ewe 803, whose lamb died when it was 24 hours old, had after 48 hours a considerably higher Se^{75}-concentration in the milk than the other ewes in A 2 (cf. ewe 5 in group A 1).

Table 1 shows the results of fractionating the whole milk from ewes 106 and 8. During the fractionation of the milk, part of the selenium was lost. Calculated on the basis of seven samples



1 a 1 b

Figure 1 a. Mean value curve for concentration of Se^{75} in whole milk from four ewes (A 1) injected with Se^{75}-sodium selenite before delivery. After 48 hours the curve represents the mean value from three sheep. Ewe 5, whose lamb was killed after 24 hours, is represented after 48 hours by a separate curve. 10,000 cpm/ml corresponds to 2.3 per cent of the injected dose per liter milk.

Figure 1 b. Mean value curve for concentration of Se^{75} in whole milk of four ewes (A 2) injected with Se^{75}-sodium selenite after delivery. Between 48 and 72 hours the mean value curve represents values from three ewes and thereafter from two. Ewe 803, whose lamb died after 24 hours, is represented by a separate curve. Ewe 103 was excluded from the curve after 72 hours when her lamb became ill. 10,000 cpm/ml corresponds to 2.3 per cent of the injected dose per liter milk.

from ewe 106, 44 ± 4 per cent remained (mean $\pm$ s), and for eight samples from ewe 8, the corresponding figure was 28 ± 13 per cent. Most of the selenium was found in the casein, which was also the largest protein fraction. The radioactivity per mg protein was lower in the casein fraction than in any of the other fractions.

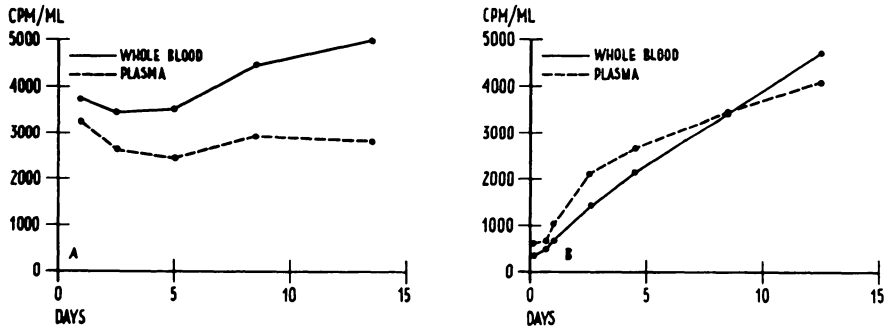
The radioactivity in whole blood and plasma (Fig. 2 a and b) as well as tissues of the lambs (Table 2) gives an approximate idea of the extent of the excretion of Se^{75} in the milk of the ewes which were injected before (A 1) or after (A 2) delivery (Fig. 2 a and b). In group A 1 the Se^{75}-concentration was high in the samples taken during the first 24 hours. The radioactivity in the whole blood, which was consistently higher than in the plasma,

Table 1. Distribution of Se^{75} among the various fractions of whole milk in per cent and in counts per minute (cpm) per mg protein. Ewe 106 was injected 12 days before delivery and ewe 8 two hours after delivery with 0.48 mg of Se^{75}-sodium selenite.

Fraction	Ewe 106		Ewe 8	
	per cent of radioactivity (mean ± s)[1])	cpm/mg protein[1])	per cent of radioactivity (mean ± s)[2])	cpm/mg protein[2])
Cream	12 ± 9	—	14 ± 5	—
Casein	66 ± 15	9	74 ± 12	27
Immune lactoglobulin I	3 ± 3	38	1 ± 1	38
Immune lactoglobulin II	4 ± 2	68	3 ± 2	75
Lactoglobulin	3 ± 1	27	3 ± 3	68
Remainder	11 ± 8	—	5 ± 4	—
Whole milk	—	16	—	46

[1]) The figures represent the mean value of 7 different samples taken during 14 days.

[2]) The figures represent the mean value of 8 different samples taken during 15 days.



2 a 2 b

Figure 2 a. Mean value curve for concentration of Se^{75} in whole blood and plasma of lambs (A 1) whose mothers were injected subcutaneously with Se^{75}-sodium selenite before delivery.

Figure 2 b. Mean value curve for concentration of Se^{75} in whole blood and plasma of lambs (A 2) whose mothers were injected with Se^{75}-sodium selenite after delivery.

increased slightly after five days. In group A 2 the Se^{75}-activity in the blood of the lambs increased and reached approximately the same level as in A 1 after 12 days. The Se^{75}-plasma concentration was higher than that in whole blood up to 8—9 days.

Table 2. Se^{75}-concentration and amount in various organs of lambs $8_2♂$, $5_1♀$ and $106_2♂$ in cpm/g wet weight and in percentage of the administered dose. The ewes in group A 2 received Se^{75} immediately after delivery and ewes in A 1 received the same dose 12—13 days before delivery.

Lamb	$8_2♂$ (A 2)		$5_1♀$ (A 1)		$106_2♂$ (A 1)	
Body weight	4.3 kg		2.7 kg		3.0 kg	
	cpm/g	%	cpm/g	%	cpm/g	%
Kidney	40,952	0.30	30,750	0.10	25,619	0.06
Adrenal gland	14,331	—	15,624	—	11,099	—
Mesenteric lymph nodes	15,852	—	16,040	—	—	—
Thyroid gland	8,842	—	15,602	—	9,263	—
Liver	13,684	0.42	15,424	0.18	8,129	0.11
Spleen	13,808	0.04	14,334	0.01	8,434	0.03
Lymph nodes	9,924	—	8,697	—	6,230	—
Lungs	8,518	0.22	8,609	0.12	4,605	0.09
Myocardium	6,099	0.06	6,305	0.03	3,700	0.02
Pancreas	2,575	—	4,964	—	1,646	—
Skeletal muscles	1,903	—	1,726	—	1,386	—
Whole blood cpm/ml	4,307	—	4,650	—	2,922	—
Plasma cpm/ml	3,297	—	4,316	—	2,998	—

Table 2 shows the Se^{75}-concentration and Se^{75}-content in tissues of lambs ($8_2♂$, $5_1♀$, $106_2♂$). The ewe of lamb $8_2♂$ was injected with Se^{75} immediately after delivery and the lamb was killed 13 days later. The ewes of lambs $5_1♀$ and $106_2♂$ were injected 12 and 13 days before delivery respectively. These lambs were killed immediately after birth. The Se^{75}-content in kidneys, liver, spleen, lungs and heart of lamb $8_2♂$ was at least twice that of lamb $5_1♀$ or $106_2♂$. The results indicate that the transport of inorganic selenium to the fetus is low compared with the transport to the young via the milk.

In Fig. 3 a and b the results are given of the Se^{75}-concentration in the whole blood and plasma of ewes injected with Se^{75}-selenite (B 1) or with Se^{75}-selenomethionine (B 2) one month after delivery. On the whole, the radioactivity was on the same level in the blood of the ewes in both groups. On the other hand, those who were injected with Se^{75}-selenomethionine had a considerably higher activity in their milk (Fig. 4). The highest values were found in the samples taken six hours after injection in both groups.

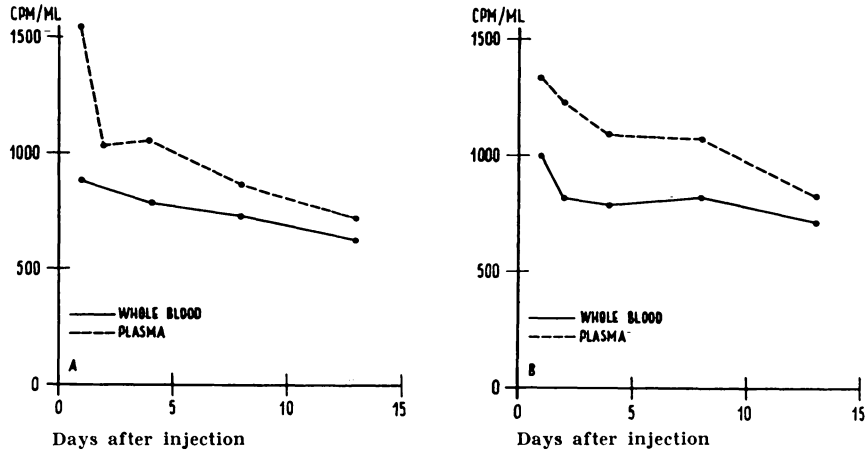


Figure 3 a. Mean value curves for concentration of Se^{75} in plasma and whole blood of two ewes (B 1) injected with Se^{75}-sodium selenite one month after delivery.

Figure 3 b. Mean value curves for concentration of Se^{75} in plasma and whole blood of two ewes (B 2) injected with Se^{75}-selenomethionine one month after delivery.

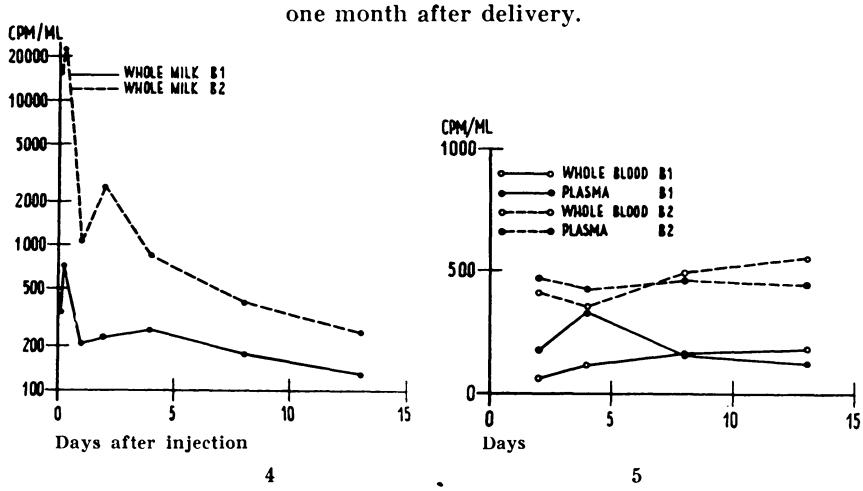


Figure 4. Mean value curves for concentration of Se^{75} in whole milk from ewes injected subcutaneously one month after parturition with Se^{75}-sodium selenite (B1, two ewes) or with Se^{75}-selenomethionine (B 2, two ewes). 1,000 cpm/ml corresponds to 3.1 per cent of the injected Se^{75}-selenomethionine and about 4.6 per cent of Se^{75}-sodium selenite per liter milk.

Figure 5. Mean value curves for concentration of Se^{75} in whole blood and plasma of lambs whose mothers were injected subcutaneously one month after delivery with Se^{75}-sodium selenite (B 1, two lambs) or with Se^{75}-selenomethionine (B 2, two lambs).

Table 3. Distribution of Se^{75} among the various fractions of the milk in per cent and in cpm/mg protein. In group B 1 the ewes were given Se^{75}-sodium selenite and in B 2 Se^{75}-selenomethionine one month after delivery.

Fraction	Group B 1		Group B 2	
	per cent of radioactivity (mean ± s)[1])	cpm/mg protein[1])	per cent of radioactivity (mean ± s)[2])	cpm/mg protein[2])
Cream	6 ± 7	—	3 ± 3	—
Casein	87 ± 8	9	83 ± 4	193
Whey	7 ± 6	9	14 ± 3	202
Whole milk	—	12	—	231

[1]) The values given are the mean value of 13 samples taken from ewes 273 and 275 during a period of 13 days.

[2]) The values given are the mean value of 14 samples taken from ewes 274 and 278 during a period of 13 days.

Milk samples from each ewe (in both groups) were fractionated as cream, casein and whey. Of the original activity in the whole milk, there subsequently remained, on an average, 69 ± 19 per cent (mean ± s) in group B 1 and 80 ± 6 per cent (mean ± s) in group B 2. Most of the Se^{75} was found in the casein fraction either the Se^{75} was administered in organic or inorganic form (Table 3). When calculated per mg of protein, the concentration of radioactivity was approximately of the same size in the various fractions and in the whole milk. The ewes that were injected with Se^{75}-selenomethionine had a specific activity of Se^{75} that was about 20 times higher than that of the ewes injected with Se^{75}-sodium selenite.

Fig. 5 shows the results of investigations of the concentration of Se^{75} in whole blood and plasma of lambs. The mothers were injected with Se^{75}-sodium selenite (B 1) or Se^{75}-selenomethionine (B 2) one month after delivery. As is evident from the mean value curves, the lambs in B 2 had a higher Se^{75}-concentration in both the whole blood and the plasma than did the lambs in B 1.

DISCUSSION

When Se^{75}-selenomethionine was administered, the concentration of radioactivity in the milk was markedly higher (about 20 times) than when inorganic selenium was given. The radioactivity was mainly bound to the protein fraction. These two findings may indicate that the selenomethionine is directly in-

corporated into the lactoprotein molecules as a normal amino acid. This assumption is also supported by earlier findings where it was observed that selenomethionine was incorporated in pancreatic proteins (*Hansson & Blau* 1963). That the accumulation of selenomethionine is high in the mammary glands was demonstrated in an autoradiographic study on the distribution of selenomethionine in the mouse (*Hansson & Jacobsson* 1965).

Also after the administration of radioactive selenium in an inorganic form, most of it was bound to the lactoproteins. When the milk was fractionated, however, 30—70 per cent of its Se^{75} was lost. This indicates that a large part of the selenium was not incorporated into the lactoproteins, but occurred as a compound which readily evaporated. In ewe 106, which was injected 13 days before delivery, considerably more Se^{75} was retained after fractionation than in ewe 8, which was injected immediately after delivery. This indicates that a large part of the selenium in the latter ewe was not as firmly bound to protein. *McConnell & Roth* (1964) consider that inorganic selenium which was administered to dogs was converted into organic selenium, since nearly all of it was bound to lactoprotein. According to *Rosenfeld & Eppson* (1964) it is probable that the rumen microorganisms must take part in the conversion of inorganic selenium to organic, before it can be incorporated into the proteins. It is therefore conceivable that a large part of the selenium in ewes injected 13 days before delivery had had time to be converted into an organic form in the rumen and was subsequently incorporated into the lactoproteins. The attachment of selenium to the lactoproteins, which occurred in ewes only a few hours after injection of inorganic selenium is apparently a different kind of bond from that which occurs with selenoamino acids.

The distribution of Se^{75} in the various protein fractions of ewe's milk is in agreement with *McConnell*'s (1948) result in rat. Most of the selenium was present in the casein fraction and the distribution was more or less proportional to the size of the fractions.

Already two hours after a subcutaneous, subtoxic injection of inorganic Se^{75} into two bitches, *McConnell & Roth* (1964) were able to demonstrate the isotope in the milk. They obtained the maximal Se^{75}-content in milk in the two bitches after four and five days respectively. Our investigations showed that in the ewes injected with selenite after delivery, the presence of radioactive

selenium was demonstrated after two hours as in the bitches. The highest concentration of Se^{75} was, however, obtained in sheep 24 hours after injection. If the injection was made before delivery, the concentration in milk was the highest in those samples which were taken less than six hours after delivery. The Se^{75}-content was almost proportional to the protein content of these samples.

The amount of selenium excreted in the milk can be approximately calculated on the basis of *Owen*'s investigations (1955). He showed that ewes of Welsh Mountain breed, during the first and second week after parturition, yielded 1043 g of milk per 24 hours, and during the fourth and fifth week 816 g per 24 hours. It may be assumed that the measurements of the Se^{75}-concentration in the whole milk in two successive samples, represent the concentration in the milk excreted during the interval between the taking of the samples. Of the selenite dose which was administered before delivery, approximately 7 per cent was excreted via the milk during the first two weeks following delivery. In ewes which were injected after delivery, about 12 per cent of the selenite dose and 62 per cent of the selenomethionine dose were excreted within two weeks after injection. In lactating rats *McConnell* (1948) found 2.5—9.3 per cent in the young 24 hours after one or more subcutaneous injections of selenate; this agrees with the values we have obtained for the ewes.

Jones & Godwin (1963) showed that a mouse fed organic selenium compounds transmitted within 24 hours 20 per cent of the activity to her young via the milk. On the whole, this corresponds to the estimated Se^{75}-excretion via the milk, during the first 24 hours in ewes injected with Se^{75}-selenomethionine.

When comparing lambs of ewes injected before and after delivery it is apparent that at least twice as much selenium is transferred to the lambs via the milk than via the placenta. The low content in newborn lambs is to a great extent due to the placental barrier for selenium (*Wright & Bell* 1964).

The present results support *Hartley*'s (1961) recommendation that it is appropriate to treat ewes one month before the estimated time of delivery. A considerable transport of selenium to the fetus then occurs and congenital muscle degeneration may be prevented. In order to maintain the concentration of selenium in the milk and thus satisfy the lamb's needs, the ewe can be treated again during the first or second week after delivery.

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SUMMARY

The excretion of selenium in milk was investigated in twelve ewes. Four of these were injected 12—29 days before, and four immediately after delivery, with Se^{75}-sodium selenite. One month after delivery two ewes were injected with Se^{75}-selenomethionine, and two with Se^{75}-sodium selenite. The Se^{75}-concentration was measured in the milk and its various fractions as well as in the plasma, whole blood and organs of three lambs.

The excretion of Se^{75} into the milk was markedly higher when Se^{75}-selenomethionine was administered than when Se^{75}-sodium selenite was given. The amount of Se^{75} in the various milk fractions was, to a great extent, proportional to the amount of protein in the fractions; this applied to the administration of organic as well as inorganic selenium. The highest Se^{75}-concentration in the milk was obtained already within 24 hours after injection. Selenium administered before delivery gave the highest concentration in samples taken less than six hours after delivery. The lambs which received Se^{75} via the milk had a higher content of Se^{75} than those who received it during the fetal period.

ZUSAMMENFASSUNG

Die Ausscheidung von Selenium in der Milch von Schafen.

Die Ausscheidung von Selen mit der Milch wurde bei 12 Mutterschafen untersucht. Vier von diesen wurde Se^{75}-Natriumselenit 12—29 Tage vor dem Partus und vier Mutterschafen unmittelbar nach demselben injiziert. Einen Monat nach dem Partus erhielten zwei Mutterschafe Se^{75}-Selenmethionin und zwei Mutterschafe Se^{75}-Natriumselenit injiziert. Der Gehalt an Se^{75} wurde ausser in Vollmilch und in deren verschiedenen Fraktionen, im Vollblut, Plasma und in Organen bestimmt.

Die Ausscheidung von Se^{75} mit der Milch war nach der Zufuhr von Se^{75}-Selenmethionin markant höher als nach der Verabreichung von Se^{75}-Selenit. Der Gehalt verschiedener Fraktionen an Se^{75} verhielt sich zum grossen Teil proportional zum Eiweissgehalt der entsprechenden Fraktion, gleichgültig ob dasselbe in organischer oder anorganischer Form gegeben wurde. Die höchste Se^{75}-Konzentration in der Milch wurde schon innerhalb von 24 Stunden nach der Injektion nachgewiesen. Wurde dieselbe vor dem Partus ausgeführt, so erreichte die Konzentration ihr Maximum in den Proben, welche früher als sechs Stunden nach dem Partus entnommen worden waren. Die Übertragung von Se^{75} auf das Lamm in der entsprechenden Zeit war auf dem Wege der Milch grösser als auf dem Wege der Plazenta.

SAMMANFATTNING

Utsöndring av selen i mjölken hos får.

Utsöndringen av selen med mjölken undersöktes hos 12 tackor. Fyra av dessa injicerades 12—29 dygn före och fyra strax efter förlossningen med Se^{75}-natriumselenit. En månad efter förlossningen injicerades två tackor med Se^{75}-selenmetionin och två med Se^{75}-natriumselenit. Halten Se^{75} mättes utom i helmjölk och dess olika fraktioner i helblod, plasma och organ.

Utsöndringen av Se^{75} i mjölken var markant högre vid tillförsel av Se^{75}-selenmetionin än vid tillförsel av Se^{75}-selenit. Halten Se^{75} i olika fraktioner var till stor del proportionell till äggvitehalten i respektive fraktion oavsett om det tillfördes i organisk eller oorganisk form. Högsta Se^{75}-koncentrationen i mjölken påvisades redan inom 24 timmar efter injektionen. Om denna gjordes före förlossningen var koncentrationen högst i proven som togs mindre än sex timmar efter partus. Överföringen av Se^{75} till lammen var större via mjölken än via placenta under motsvarande tid.

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