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THE PLACENTAL TRANSMISSION OF SELENIUM IN SHEEP*)

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It has been shown that small amounts of selenium have a protective effect against white muscle disease and give a positive growth response in lambs when it is supplied to pregnant ewes (*Muth et al.* 1958, 1959; *McLean et al.* 1959; *Oldfield et al.* 1960). On the other hand malformed eyes and legs in lambs of ewes that grazed on seleniferous areas have been reported (*Beath et al.* 1939; *Rosenfeld & Beath* 1947).

Selenium is transmissible through the placenta to the mammalian fetus whether supplied as inorganic or as organic compounds (*Westfall et al.* 1938; *McConnell & Roth* 1964; *Rosenfeld* 1964; *Hansson & Jacobsson* 1966).

In sheep limited information is available on the quantitative placental transmission of selenium to the fetuses when supplied to the ewes in various amounts and in various chemical forms. *Burton et al.* (1962) showed that lambs born to ewes given injections of selenium had an increased tissue selenium level, and *Wright & Bell* (1964) found Se^{75} in the fetus of ewes provided with radioselenium orally as H_2SeO_3. The concentration in the fetuses was, however, lower than in the ewes indicating a placental barrier for selenium.

The purpose of this investigation was to study the placental transmission of selenium during late gestation in ewes injected with Se^{75}-selenomethionine, Se^{75}-selenocystine and Se^{75}-sodium selenite.

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MATERIAL AND METHODS

Radioactive selenium compounds: Se^{75}-sodium selenite and Se^{75}-(L)-selenomethionine were obtained in aqueous solution from The Radiochemical Centre, Amersham, England.

The Se^{75}-sodium selenite had at the beginning a specific activity of 0.5—0.7 mC/mg. The solution was diluted with physiological saline to a concentration of 0.096 mg of selenium per ml (corresponding to 0.05—0.07 mC Se^{75}). Non-radioactive sodium selenite was added to get a higher dose of selenium in one series of animals.

The specific activity of the Se^{75}(L)-selenomethionine was at the beginning 90 mC/mM. The solution was diluted with physiological saline and non-radioactive DL-selenomethionine from Calbiochem, Los Angeles, USA, was added so that 2.0 ml contained 0.47 mg of selenomethionine (corresponding to 0.06 mC Se^{75}).

Se^{75} (L)-selenocystine was obtained from Farbwerke, Hoechst AG, Germany. The specific activity was 0.21 μC/mg. 238 mg of the substance (corresponding to 50 μC Se^{75}) was dissolved in 100.0 ml 0.1 N-HCl.

Animals: The animals were of Swedish "lantras" type, weighing between 34 and 62 kilos and varying in age from two to ten years. During the experimental period the animals received conventional fodder.

Four ewes were injected subcutaneously 12—29 days before delivery with 5.0 ml of a solution containing 0.48 mg of selenium (corresponding to 0.35 mC Se^{75}) in the form of Se^{75}-sodium selenite. These ewes had six lambs and three of them were killed soon after birth. The radioactivity in the organs of the lambs was compared with that in two rams injected with the same dose and killed 13 days thereafter.

Five ewes were injected subcutaneously with 2.0 ml solution containing 5.1 mg of selenium (corresponding to 0.05 mC Se^{75}) in the form of Se^{75}-sodium selenite 2—22 days before delivery. The ewes and the five lambs were killed soon after delivery.

Three ewes were injected subcutaneously with 2.0 ml solution containing 0.47 mg of Se^{75}-selenomethionine 2—24 days before delivery. The ewes and their three lambs were killed soon after delivery.

One ewe was injected with 50.0 ml solution containing 119 mg of Se^{75}-selenocystine intravenously 18 days before delivery. The ewe and her lamb were killed soon after delivery.

Soon after delivery, blood samples were taken from ewes and lambs. When the blood samples were taken the animals, which were to be killed, were anesthetized with pentobarbital sodium and bled to death. The weights of kidneys, liver, spleen, lungs and heart were determined. Tissue samples (approximately 1 g) were taken for radioactive measurements. The volume of the whole blood was calculated in adults as 80 ml per kilo and in lambs as 166 ml per kilo (*Dukes*

Table 1. Se^{75}-concentration in various tissues of adults and lambs. The adults were injected s.c. with Se^{75}-sodium selenite.

Per cent of dose per gram wet tissue $\times 10^3$

Dose	0.48 mg Se						5.1 mg Se					
Animals	Adults[b]			Lambs			Adults			Lambs		
	n	$\bar{x}$	range	n	$\bar{x}$	range	n	$\bar{x}$	range	n	$\bar{x}$	range
Whole blood[a]	6	2.1	1.8— 2.3	6	1.0	0.5— 1.7	5	1.6	1.5— 1.9	5	0.8	0.4—1.5
Plasma[a]	6	2.1	1.3— 2.6	6	0.9	0.4— 1.4	5	1.7	0.9— 2.5	5	0.6	0.5—0.6
Kidney	2	38.6	28.6—48.5	3	7.7	6.0—10.0	5	11.6	8.1—17.0	5	3.2	2.3—4.4
Liver	2	6.5	2.8— 9.2	3	3.1	1.9— 3.7	5	11.4	3.5—21.9	5	2.0	1.6—2.7
Lung	2	4.2	3.6— 4.8	3	1.5	1.1— 2.0	5	2.9	1.9— 4.5	5	0.9	0.6—1.3
Adrenal	2	10.8	9.6—12.0	3	4.3	2.6— 6.5	5	2.8	2.6— 3.1	5	2.0	1.1—3.3
Pancreas	2	8.1	6.7— 9.5	3	1.2	0.4— 2.0	5	2.9	2.5— 3.3	5	1.4	1.0—1.9
Spleen	2	6.4	5.9— 7.0	3	3.3	2.0— 4.6	5	2.5	2.1— 3.1	5	1.5	1.0—2.2
Thymus							5	1.4	1.2— 1.6	5	1.2	0.7—1.6
Lymph nodes	2	6.8	6.6— 7.0	3	2.0	1.5— 2.6	5	2.2	1.9— 3.4	5	1.6	0.7—3.5
Abomasum	2	2.0	1.3— 2.6	3	1.9	1.6— 2.0	5	1.0	0.8— 1.3	4	0.9	0.7—1.2
Skel. muscle	2	0.9	0.8— 1.0	3	0.5	0.3— 0.7	5	0.3	0.2— 0.5	5	0.3	0.2—0.6
Myocardium	2	4.4	4.0— 4.9	3	1.5	0.9— 2.3	5	1.8	1.5— 2.0	5	0.9	0.6—1.2
Brain							3	0.6	0.4— 0.7	3	0.5	0.2—0.9
Milk[a]	3	1.7	0.5— 2.3				5	1.3	0.7— 1.9			

a) Expressed per ml b) This data is taken from two rams killed 13 days after injection and from four ewes

1947) and the weight of skeletal muscles as 29 per cent*) of the body-weight.

Measurement of radioactivity: The radioactivity was measured with a well-type scintillation detector connected to a single-channel analyzer and a scaler. Corrections were made for radioisotope decay.

RESULTS

The distribution of Se^{75} in adult sheep and lambs after subcutaneous injection of Se^{75}-sodium selenite is shown in Table 1. The concentration of Se^{75} per gram of tissue was with a few exceptions higher in the group receiving the lower dose of selenium.

The Se^{75}-concentration in most tissues of lambs was about half that of the adult regardless of the dose. The difference was

Table 2. Distribution of Se^{75} in tissues of lambs born by ewes injected s.c. with 0.48 mg or 5.1 mg of Se as Se^{75}-sodium selenite before delivery.

Per cent of dose per gram wet tissue $\times 10^3$

Dose	0.48 mg Se			5.1 mg Se				
Injection[a])	12	13	29	2	8	8	17	22
	Single lamb	Twins	Twins	Single	Single	Single	Single	Single
Whole blood[b])	1.1	0.7	1.7	0.4	0.7	0.7	1.5	0.9
Plasma[b])	1.0	0.7	1.4	0.6	0.5	0.6	0.6	0.5
Kidney	7.2	6.0	10.0	2.3	2.9	2.9	4.4	3.5
Liver	3.6	1.9	3.7	1.6	1.9	1.8	2.7	1.9
Lung	2.0	1.1	1.4	0.7	1.2	0.9	1.3	0.6
Adrenal	3.7	2.6	6.5	1.1	3.3	1.5	2.4	1.7
Pancreas	1.2	0.4	2.0	1.0	1.4	1.4	1.9	1.2
Spleen	3.4	2.0	4.6	1.0	1.5	1.1	2.2	1.9
Thyroid	3.7	2.2	6.6	0.9	1.0	1.0	1.6	1.5
Thymus	—	—	—	0.7	1.4	1.0	1.6	1.5
Lymph nodes	2.0	1.5	2.6	0.7	1.2	1.1	3.5	1.5
Abomasum	2.0	1.6	2.1	0.7	1.2	1.0	—	0.8
Skel. muscle	0.4	0.3	0.7	0.2	0.3	0.2	0.6	0.3
Myocardium	1.5	0.9	2.3	0.6	1.0	0.8	1.2	1.1
Brain	—	—	—	0.2	0.4	—	—	0.9

a) Time for injection in days before delivery

b) Expressed per ml

*) Information from S.G.S., the Stockholm-Gävle Slaughterhouse Association.

greatest in the kidney. In the abomasum wall of the lambs the Se^{75}-accumulation was as high as in the adults. The same was the case with the skeletal muscles, the thymus and the brain in the higher dosed group.

The lamb born two days after the injection of the ewe had lower Se^{75}-content than lambs born after 8—22 days. The Se^{75}-concentration in the lamb born 29 days after the injection was slightly higher than in those born after 12 and 13 days (Table 2). The single lamb born 12 days after the injection had a greater percentage of the Se^{75}-dose than the twin lamb born after 13 days.

The amount of the dosed Se^{75}-sodium selenite in some organs of lambs is shown in Table 3. The mean birth-weight in the higher dosed group, with only single lambs was 0.4 kg higher than in the other group, in which two of three were twins. The skeletal muscles, the blood and the liver had the highest amount of the Se^{75}-dose. The total content of Se^{75} in seven organs was 1.0 per cent of the dose when 5.1 mg Se was given to the ewes and 1.3 when 0.48 mg Se was given.

The Se^{75}-concentration in various organs of ewes and lambs after injection of Se^{75}-methionine or Se^{75}-selenocystine is shown in Table 4. In a few organs the ewes had a higher Se^{75}-concentration than the lambs. The thymus, the skeletal muscles and the abomasum wall had, however, lower values in the selenomethionine injected ewes than in their lambs. The Se^{75}-accumu-

Table 3. The Se^{75}-amount in various organs of lambs born by ewes injected with Se^{75}-sodium selenite before delivery.

Per cent of dose in the whole organ

Dose	0.48 mg Se[a]			5.1 mg Se[b]		
	n	$\bar{x}$	range	n	$\bar{x}$	range
Whole blood	3	0.54	0.34—0.78	5	0.42	0.21—0.70
Skel. muscles	3	0.40	0.28—0.59	5	0.29	0.19—0.47
Liver	3	0.15	0.11—0.18	5	0.14	0.10—0.17
Kidneys	3	0.09	0.06—0.11	5	0.06	0.05—0.08
Lungs	3	0.09	0.07—0.12	5	0.06	0.04—0.08
Myocardium	3	0.03	0.02—0.04	5	0.03	0.02—0.04
Spleen	3	0.02	0.01—0.03	5	0.01	0.01—0.01
Sum	–	1.32	0.89—1.85	–	1.01	0.62—1.55

a) Mean birth-weight was 2.8 kg
b) Mean birth-weight was 3.2 kg

Table 4. Se^{75}-concentration in various tissues of ewes and lambs. The ewes were administered Se^{75}-selenomethionine or Se^{75}-selenocystine 2—24 days before delivery.

Per cent of dose per gram wet tissue $\times 10^3$

Dose	0.47 mg Se^{75}-selenomethionine						119 mg Se^{75}-selenocystine			
Animal	Ewes			Lambs			Ewe		Lamb	
	n	$\bar{x}$	range	n	$\bar{x}$	range	n	x	n	x
Whole blood[a])	3	2.7	2.4— 2.9	3	2.2	1.2— 3.8	1	2.3	1	1.3
Plasma[a])	3	2.9	1.9— 4.3	3	2.2	1.7— 2.6	1	2.0	1	1.2
Kidney	3	38.6	23.7—52.7	3	19.3	12.8—29.3	1	11.9	1	9.6
Liver	3	7.6	5.3—10.1	3	9.1	7.8—11.2	1	6.0	1	3.7
Lung	3	4.5	3.1— 5.4	3	4.0	2.9— 5.9	1	4.1	1	2.0
Adrenal	3	16.9	10.3—21.8	3	12.1	8.2—16.9	1	10.0	1	4.0
Pancreas	3	22.6	14.9—31.0	3	10.5	8.6—13.1	1	10.3	1	2.8
Spleen	3	7.0	4.2— 8.7	3	7.0	4.8—10.8	1	5.0	1	4.0
Thymus	3	5.2	3.8— 5.9	3	7.6	5.6—11.0	1	—	1	3.0
Lymph nodes	3	6.9	6.2— 7.3	3	6.8	4.3—11.3	1	1.1	1	1.7
Abomasum	3	4.3	3.4— 6.0	3	5.9	4.2— 7.1	1	2.6	1	2.9
Skel. muscle	3	0.9	0.7— 1.1	3	2.7	1.9— 3.2	1	0.8	1	0.8
Myocardium	3	3.4	3.0— 4.0	3	4.4	3.9— 5.1	1	2.5	1	2.0
Brain	2	4.3	3.2— 5.4	2	6.4	3.1— 9.7	1	2.8	1	2.1
Milk[a])	3	5.9	1.3—12.6				1	2.1		

a) Expressed per ml

lation was highest in the pancreas, the kidneys and the adrenals of ewes and lambs after injection of both substances. The amount of Se^{75} in some organs of the lambs is listed in Table 5. The skeletal muscles had the greatest percentage of the Se^{75}-dose. The total amount found in these organs was 4.8 per cent after injection of Se^{75}-selenomethionine and 1.9 per cent after injection of Se^{75}-selenocystine.

DISCUSSION

Our investigation has shown that the concentration of Se^{75} in most organs of the fetus was lower than in the mother. When the ewes were injected with Se^{75}-sodium selenite the concentration in the blood of the adults was twice that of the lambs. This indicates that the placenta, as earlier has been stated (*Wright & Bell* 1964; *Jacobsson & Hansson* 1965), constitutes to a certain

Table 5. The Se^{75}-amount in various organs of lambs born by ewes injected with Se^{75}-selenomethionine or Se^{75}-selenocystine before delivery.

Per cent of dose in the whole organ

Dose	0.47 mg Se^{75}-selenomethionine[a]			119 mg Se^{75}-selenocystine[b]	
	n	$\bar{x}$	range	n	x
Whole blood	3	1.11	0.65—1.91	1	0.65
Skel. muscle	3	2.34	1.75—2.83	1	0.68
Liver	3	0.63	0.43—0.78	1	0.23
Kidneys	3	0.34	0.22—0.51	1	0.15
Lungs	3	0.21	0.15—0.30	1	0.08
Myocardium	3	0.11	0.09—0.14	1	0.06
Spleen	3	0.03	0.02—0.04	1	0.02
Sum	–	4.77	3.31—6.51	–	1.87

a) Mean birth-weight was 3.3 kg
b) Birth-weight was 3.0 kg

extent a barrier for the passage of inorganic selenium from the mother to the fetus.

The reason why the transmission of selenium to the fetus is limited may depend on the apparent fixation or binding of selenium to the blood proteins. In the dog a certain amount of selenium exists in a fairly stable protein-like combination (*McConnell & Cooper* 1950). Time-distribution investigations have shown that selenium is bound principally to albumin during the first hour, then later transferred and principally bound to the α- and β-globulin fractions (*McConnell & Levy* 1962). The movement of $Se^{75}O_3^{=}$ from the mother to the fetus is probably a diffusion. Since very little unbound selenium is available the transport to the fetus will be small. The results seem to indicate that the transport via the placenta is high during the first days after administration. This is very likely due to a higher degree of non-protein-bound selenium which the excretion pattern via the urine indicates.

Another reason for the difference in Se^{75}-concentration between the maternal and the fetal tissues may depend on differences in water and protein content between these tissues. Most fetal tissues have a lower dry weight and concentration of protein than the corresponding maternal tissues (*Carlyle* 1945; *McCance & Widdowson* 1961). Since the selenium to a large extent is

associated with proteins we can assume that this factor plays a part. The ratio, however, between the dry weight of lamb and mother is only approximately 3:4 while our results show a ratio between Se^{75}-concentration of 1:5 for the liver and the kidneys. We therefore assume that the placental barrier, the differences in dry weight and protein content contribute to the existing difference.

When the ewes were injected with Se^{75}-selenomethionine, or Se^{75}-selenocystine, the Se^{75}-concentration in the lambs was nearly as high as in the mother. The same was reported in mice injected with Se^{75}-selenomethionine (*Hansson & Jacobsson* 1966). In rats *Westfall et al.* (1938) also showed that more selenium is stored in the fetus when the mother's diet contains naturally occurring organic food selenium than when it contains sodium selenite. It is evident from this and other investigations (*Hansson & Blau* 1963; *Hansson* 1963) that selenomethionine behaves like the sulphur analogue methionine. It has been suggested that the natural amino acids cross the cell and placental membranes against a concentration gradient (*Widdas* 1961). The Se^{75}-selenomethionine seems to have the same properties since it is actively transported through the intestinal wall in competition with methionine (*McConnell & Gloria* 1965).

Wright & Bell showed that the distribution of the radioselenium pattern in fetal tissues was similar to that in maternal tissues and that Se^{75}-concentration in the single fetus was twice that of twins. Our findings are in agreement with their results.

The present results show that when the doses of Se^{75}-sodium selenite were increased ten times (0.48 mg Se to 5.1 mg Se) the uptake of Se^{75} per gram tissue in the lambs was increased five to eight times. That the increase was not ten times can be explained by the more rapid excretion of selenium at the higher dose level.

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SUMMARY

The placental transmission of selenium was investigated in thirteen ewes during late gestation. The ewes were injected either with Se^{75}-sodium selenite, Se^{75}(L)-selenocystine or Se^{75}(L)-selenomethionine. Twelve lambs and nine ewes were killed soon after delivery and the Se^{75}-concentration was measured in the tissues of the lambs and ewes.

The Se^{75}-concentration in the tissues of the lambs was about half that of the ewes when they were injected with selenite. This indicates that the placenta constitutes to a certain extent a barrier for the passage of inorganic selenium. Se^{75}-selenomethionine and Se^{75}-selenocystine were more easily transferred across the placenta than Se^{75}-sodium selenite. When the selenite dose was increased ten times the selenium uptake in milligrams per gram tissue in the lambs increased 5—8 times.

ZUSAMMENFASSUNG

Die Übertragung von Selen durch die Plazenta auf den Fötus beim Schaf.

Die Übertragung des Selens auf den Fötus wurde bei dreizehn hochträchtigen Mutterschafen untersucht. Den Mutterschafen wurde entweder Se^{75}-Natriumselenit (zwei verschiedene Dosen: 0,48 oder 5,1 mg Se), Se^{75}-Selencystin oder Se^{75}-Selenmethionin subkutan injiziert. Zwölf Lämmer und neun Mutterschafe wurden gleich nach dem Partus getötet und die Se^{75}-Konzentration in den Geweben der Lämmer und der Mutterschafe gemessen.

Als den Mutterschafen Selenit injiziert wurde, betrug die Se^{75}-Konzentration in den Geweben der Lämmer ungefähr die Hälfte der in den Geweben der Mutterschafe vorhandenen. Dies deutet darauf hin, dass die Plazenta ein gewisses Hindernis für die Passage anorganischen Selens von der Mutter zum Fötus bildet. Se^{75}-Selenmethionin und Se^{75}-Selencystin passierten die Plazenta leichter als Selenit. Die Se^{75}-Konzentration bei den Lämmern war hier in den meisten Organen ebenso hoch und in gewissen Organen sogar noch höher als bei den Mutterschafen.

Wurde die Selenitdosis zehnfach erhöht, so nahm der Selenzuschuss in den Geweben der Lämmer fünf- bis achtmal zu, in Milligram Se pro Gramm Gewebe gerechnet.

SAMMANFATTNING

Överföring av selen via placenta hos får.

Överföringen av selen till foster undersöktes på tretton högdräktiga tackor. Tackorna injicerades subkutant antingen med Se^{75}-natriumselenit (två olika doser: 0,48 eller 5,1 mg Se), Se^{75}-selencystin eller Se^{75}-selenmetionin. Tolv lamm och nio tackor avlivades strax efter förlossningen och Se^{75}-koncentrationen mättes i lammens och tackornas vävnader.

När tackorna injicerades med selenit var Se^{75}-koncentrationen i lammens vävnader omkring hälften av den i tackornas. Detta tyder på att placentan utgör ett visst hinder för det oorganiska selenets passage från moder till foster. Se^{75}-selenmetionin och Se^{75}-selencystin passerade placentan lättare än selenit. Se^{75}-koncentration hos lammen var då i de flesta organ nästan lika hög som och i vissa organ högre än hos tackorna.

När selenitdosen ökade tio gånger ökade selentillskottet i lammens vävnader fem till åtta gånger räknat i milligram Se per gram vävnad.

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