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DISTRIBUTION OF SELENIUM IN MICE STUDIED BY WHOLE-BODY AUTORADIOGRAPHY AFTER INJECTION OF SE^{75}-SODIUM SELENITE*)

By

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It has been reported in recent years that selenium has an important nutritional function in many animal species (*Schwarz & Foltz* 1957; *Schultze* 1960). The mechanism by which selenium acts and the metabolic active selenium compounds are unknown. It has, however, been shown that various organic and inorganic selenium compounds have therapeutic effects in certain contexts.

Several authors have studied the distribution of inorganic and organic selenium compounds in the tissues by conventional chemical analysis (*Dudley* 1936; *Smith et al.* 1937, 1938; *Rosenfeld & Beath* 1945) and also by use of radioactive tracer (*McConnell* 1941; *Jones & Godwin* 1963).

It has been shown that selenium is widely distributed throughout the body in experiments with toxic doses (*Smith et al.* 1937; *Miller & Williams* 1940; *Maag et al.* 1960). The highest concentration was found in the liver, kidney, spleen, pancreas, heart and lungs. When supplied in tracer doses, the distribution in the body seems to be a little changed. *Buescher et al.* (1961) found the highest concentration in the kidney with relatively decreasing amount seen in liver, spleen, lungs and heart. Similar results have been observed by e.g. *Kuttler et al.* (1961), *Cousins & Cairney* (1961), *Grant et al.* (1961) and *Orstadius & Åberg* (1961).

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The present investigation is the first in a series of experiments in which we have studied the fate of selenium compounds.

We have studied the distribution of Se^{75} after administration of Se^{75}-sodium selenite with an autoradiographic technique.

Se^{75} emits internal conversion electrons which have been shown to be useful for autoradiography (*Forberg et al.* 1964). The autoradiographic technique used in the present work has the advantage of giving a general survey of the distribution of the isotope in the entire body. Pregnant animals were used in our investigation with the aim of studying the distribution of the selenium in the female and fetus at various times after the administration.

METHODS

The Se^{75}-sodium selenite was obtained from The Radiochemical Centre, Amersham, England. The specific activity was 1.14 mC/mg selenium. It was in an aqueous solution. Before injection the solution was diluted with physiological saline to a concentration of 0.096 mg selenium and a radioactivity of 0.109 mC per ml. Of this solution 0.5 ml was injected intravenously in 8 male and 8 female albino mice with a body weight of 20—25 g. One animal of each sex was killed at 5 minutes, 20 minutes, 1 hour, 4 hours and at 1, 2, 4 and 8 days after the injection. Two pregnant mice were injected with the same dose and killed after 4 hours and 2 days respectively.

The autoradiographic procedure as described by *Ullberg* (1954, 1958) was used. The mice were anesthetized with ether and then immersed in a mixture of hexane and solid carbon dioxide at about —70°C. Sagittal 20 μ and 100 μ sections through the whole animals were then taken at —10°C. Autoradiographic exposure was made by apposition against Industrex X-ray film.

RESULTS

General observations. The distribution pattern after intravenous injection of Se^{75}-sodium selenite was characterized by a high blood level of selenium during the observation period. Those organs which after 5 minutes accumulated a higher concentration of selenium than the blood were the liver, the heart, the kidney and the adrenal cortex. The results from the two series of animals were consistent. The distribution patterns at various times after the intravenous injection are shown in Figures 1—7 and are

semiquantitatively recorded in Table 1. A detailed description of selenium concentration in some organs and tissues follows.

The circulatory organs. The myocardium accumulated selenium to a very high extent, and a concentration almost as high as in the liver and the kidney (Fig. 1 and Fig. 2) was noticed already 5 minutes after the injection. Twenty-four hours after the injection the concentration in the myocardium had decreased and was only slightly above that in the blood. The content of selenium seemed to be even in various parts of the myocardium.

A slight concentration in the large blood vessel walls was also observed (Fig. 1).

The respiratory organs. The lung accumulated selenium to a certain extent in the first hours after injection. The activity was slightly higher than that of the blood. The nasal mucosa showed accumulation which was most pronounced the first hours after the injection.

The gastro-intestinal tract. Some accumulation of selenium was seen in the mucous membrane of the intestine and stomach. Activity appeared in the intestinal contents.

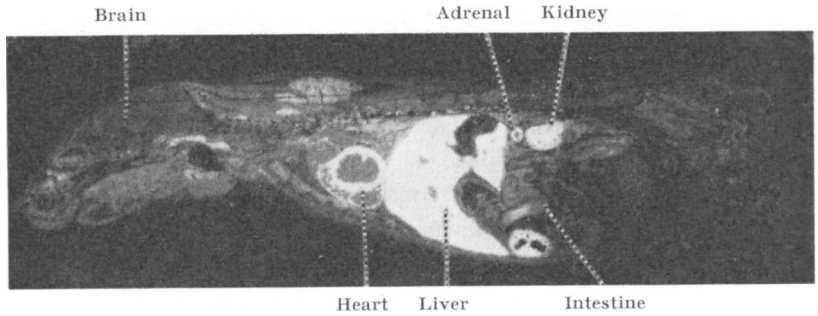
The pancreas showed a concentration which was slightly lower than the blood during the whole observation period. The salivary glands contained a rather low concentration of selenium during the first hour but the radioactivity increased and a considerable amount was seen 4 and 24 hours after the injection (Fig. 3 and Fig. 4).

The liver. Accumulation of selenium reached high levels. The concentration was considerably higher in the liver than in the blood within 5 minutes and remained so until 48 hours after the injection.

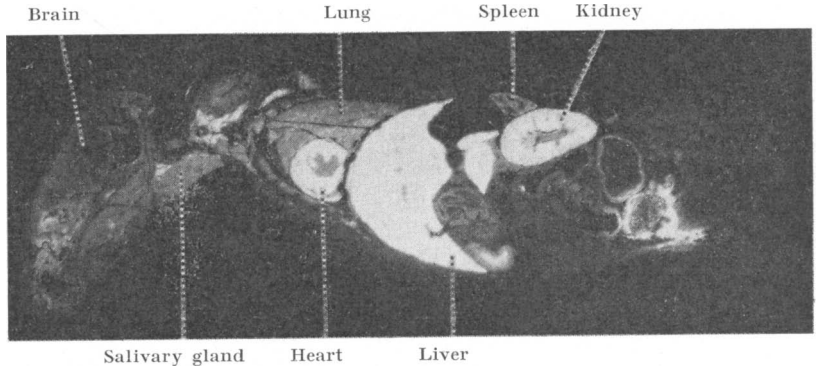
The activity was almost homogeneously spread in the liver but the liver became sometimes marbled. A concentration was seen in the gall bladder during the first hours. In animals sacrificed 48 hours after the injection and later the concentrations in the blood and the liver were approximately the same.

The central nervous system. Very low activity was seen in the brain and the spinal cord.

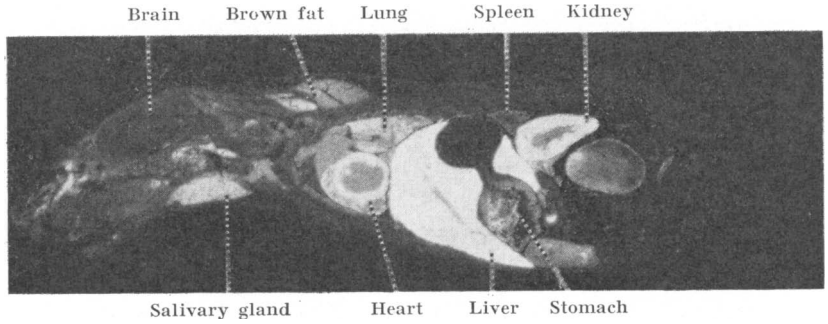
The endocrine glands. The adrenal cortex accumulated selenium (Fig. 1 and Fig. 6). By 5 minutes after the injection the concentration there was highest of all organs next the liver, the kidney and the heart. The radioactivity remained high during



F i g. 1. Autoradiogram showing the distribution of Se^{75} 20 minutes after intravenous injection of sodium selenite. White areas correspond to high level of radioactivity. Note high concentration in the liver, the heart, the kidney and the adrenal cortex.



F i g. 2. Autoradiogram showing the distribution of Se^{75} 1 hour after intravenous injection of sodium selenite. White areas correspond to high level of radioactivity. High amount of radioactivity is seen in the heart, the liver, the kidney and the urinary bladder.



F i g. 3. Autoradiogram showing the distribution of Se^{75} 4 hours after intravenous injection of sodium selenite. White areas correspond to high level of radioactivity. High concentration is seen in the liver, the kidney, the heart, the brown fat and the gastrointestinal content.

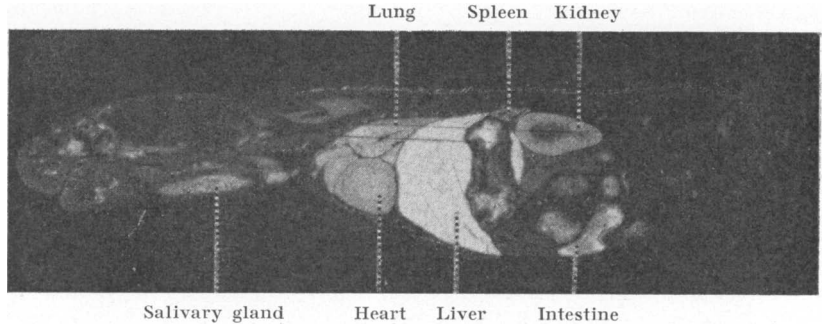


Fig. 4. Autoradiogram showing the distribution of Se^{75} 1 day after intravenous injection of sodium selenite. White areas correspond to high level of radioactivity. Note high concentration in the liver, the kidney, the salivary gland, the blood and the gastrointestinal contents.

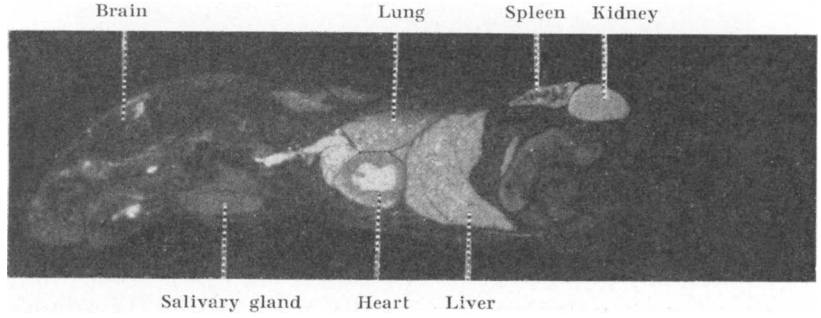


Fig. 5. Autoradiogram showing the distribution of Se^{75} 2 days after intravenous injection of sodium selenite. White areas correspond to high level of radioactivity. The highest concentration of radioactivity is seen in the blood, the liver, the kidney and the spleen.

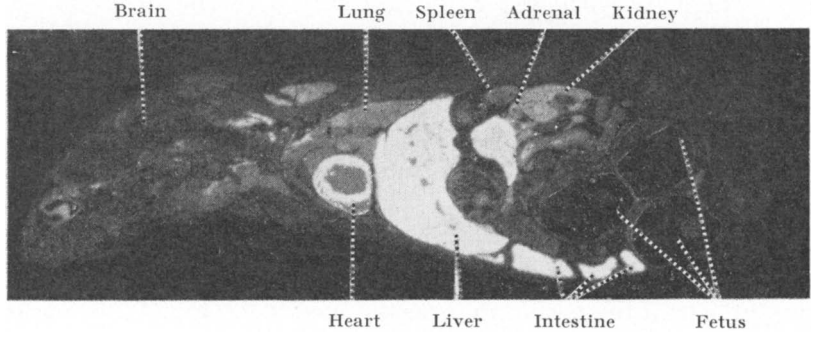


Fig. 6. Autoradiogram showing the distribution of Se^{75} in a pregnant mouse 4 hours after intravenous injection of sodium selenite. The concentration in the fetus is lower than in the dam.

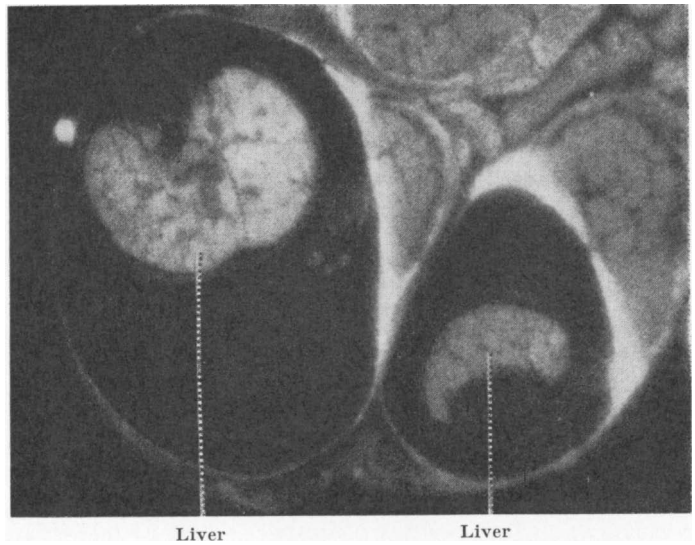


Fig. 7. Part of autoradiogram showing the distribution of Se^{75} in the placenta and two fetuses 24 hours after intravenous injection of sodium selenite in the pregnant mouse. The concentration of radioactivity in the fetus is lower than in the mother. The fetal liver showed the highest concentration of Se^{75}.

the first four hours. Very low activity was seen in the adrenal medulla.

Definite accumulation of selenium was not seen in the other endocrine organs.

The bone marrow. Some activity was seen in the bone marrow. The activity, however, did not exceed the blood level.

The lymph system. The red pulp of the spleen showed an accumulation of selenium during the whole observation period. The white pulp of the spleen had a low activity, and low activity was also seen in the thymus and the lymph nodes.

The urogenital organs. Selenium was accumulated in both the renal cortex and the renal medullae. Fig. 2 shows that after one hour a higher concentration existed in the renal medullae than in the cortex. After 24 hours the distribution of radioactivity was higher in the cortex than in the medullae (Fig. 4). Selenium was found in the urinary bladder from 5 minutes after injection and thereafter.

Low activity was seen in the testes and epididymis.

T a b l e 1. Radioactivity in some tissues of mice intravenously injected with Se^{75}-sodium selenite, as revealed by autoradiography.

Organ	5 min	20 min	1 hour	4 hours	1 day	2 days	4 days	8 days
Liver	++++	++++	++++	++++	+++	+++	++	++
Bile	—	++	++	++		+	—	—
Spleen	++	++	++	+	+	+	+	+
Pancreas	++	++	++	++	+	+	+	—
Gastric wall	+	+	+	+	+	+	+	—
Small int. wall	++	++	++	++	+	+	+	—
Large int. wall	++	++	++	++	+	+	+	—
Salivary gland	+	+	+	++	++	+	+	+
Kidney	+++	+++	+++	+++	++	++	++	++
Adrenal cortex	+++	+++	++	++	+	+	+	—
Testicle	+	+	+	+	+	+	+	++
Epididymis	—	—	—	—	—	—	—	+
Uterus	+	+		+	+	+	+	—
Yolk sac placenta				+++	+++			
Fetus				+	+			
Blood	+++	++	++	++	++	++	++	+
Bone marrow	++	++	++	++	++	+	+	—
Heart	+++	+++	+++	+++	++	+	+	—
Lung	+++	++	++	++	++	++	+	+
Harder's gland	+++	+++	+++	++	+	+	+	+
Brown fat	+	++	+++	+++	++	+	+	—

— = Faintly visible.
\+ = Detectable localization.
++ = Obvious localization.
+++ = Intense localization.
++++ = Very intense localization.

The ovaries and the uterus had a low concentration of radioactivity.

The skin. Very little activity was seen in the skin the first hours after the injection. After 24 hours some activity was seen in the hair and the epidermis (Fig. 4).

The skeleton. Except for the bone marrow little selenium was found in the skeleton. No or very little activity was seen in the cartilage.

Brown fat. A rather high concentration of selenium was observed in the brown fat during the whole observation period (Fig. 1, Fig. 3, Fig. 5).

Skeletal muscles. The skeletal muscles showed a very low uptake of selenium.

The fetus and the placenta. The concentration in the fetus 4 and 24 hours was considerably lower than in the dam (Fig. 6). The placenta, however, had a rather high concentration of radioactivity and apparently constitutes a certain barrier for the penetration of selenium to the fetus.

Fig. 7 shows part of an autoradiogram 4 hours after the injection where only some organs of the fetus can be seen. Those organs of the fetus which contain enough radioactivity to be determined with this method are the liver, the brain, and the lungs.

DISCUSSION

The autoradiograms obtained were of a very good quality. Some of the pictures, however, did not show any sharp contures when the film was overexposed. This may indicate that besides the internal conversion electrons some weak γ-rays also contributed to the autoradiographic picture.

Our findings mainly confirm earlier studies but also add some new information. The highest content of Se^{75} was observed in the liver, kidney, and spleen and thereby the results are in agreement with the results of *Smith et al.* (1938), using toxic doses.

There has been a number of reports stating that after administration of inorganic selenium to animals the selenium is found in the protein fraction e.g. *McConnell* (1963). Chromatographic investigations have shown that selenium is associated with organic material, presumably the analogues of amino acids cystine (cysteine) and methionine in which the selenium has replaced the sulphur (*McConnell & Wabnitz* 1957). Our results show very few similarities between the distribution of selenium and the amino acids methionine and cystine studied with the same method (*Hansson* 1959). The distribution of Se^{75}-selenomethionine also shows a different distribution compared with that of Se^{75}-sodium selenite. The organs with an extensive protein formation such as the pancreas, the intestinal, and stomach mucosa then show a very high accumulation of radioactivity (*Hansson & Jacobsson* 1965). Our results therefore indicate that very little of inorganic selenium when given to animals may be incorporated into selenoanalogues of sulphur-containing amino acids, during the period covered by this study (up to 8 days after injection).

The autoradiographic pictures showed that the liver and the kidney are the organs which accumulated most of the selenium. The concentration in the liver decreased rather rapidly. This is to a certain extent due to an excretion of selenium into the bile since high concentration in the biliary system was seen during the first hours after the injection.

It is interesting to notice this high accumulation of selenium in the liver in connection with its well-known action as a prophylactic agent against liver necrosis in rats and pigs (*Schwarz & Foltz* 1957; *Grant & Thafvelin* 1958).

A very high accumulation of selenium was noticed in the myocardium during the first 24 hours after the injection. This high accumulation has earlier been observed by e.g. *Kuttler et al.* (1961) and *Ekman et al.* (1963). It is likely that a part of the acute toxic effects of selenium can be explained by its very selective affinity to the heart muscle and the larger vessel walls.

In therapeutic experiments with pigs *Grant & Thafvelin* (1958) demonstrated that selenite has a selective morphological effect on the nutritionally induced lesions in the circulatory organs. Transudation and vascular changes in the myocardium and other tissues were prevented by treatment with sodium selenite. In other types of nutritional vascular disease, so-called exsudative diathesis in chickens, selenite has proved to be a good therapeutic agent. It seems that selenium in a low dose may have a therapeutic effect, and in a higher dose a toxic effect on the circulatory system. This seems to a certain extent to be dependent on the property of selenium to concentrate in these tissues.

The kidneys are the organs which besides the liver accumulate selenium. At a short time after the injection the concentration is higher in the liver than in the kidney, but 4 days after the injection and later the concentration is higher in the kidney. The high concentration in the kidney is to a large extent an expression of the excretion of selenium via urine (*Smith et al.* 1937). The kidney, however, has an ability to accumulate selenium more than any other tissue after administration of selenium during a long time period (*Cousins & Cairney* 1961; *Grant et al.* 1961). It is interesting to see that the kidney of pigs in some nutritional diseases as muscular degeneration has a lower concentration of selenium compared with normal animals (*Lindberg & Sirén* 1963).

Our studies show a very high accumulation of selenium in

the adrenal cortex. This accumulation has not been observed earlier and is difficult to interpret. *Rosenfeld* (1964) observed some concentration of selenium in the adrenal gland after long term administration of selenium. Toxicological and pharmacological studies have not reported any effects of selenium on the adrenocortical metabolism.

It has earlier been demonstrated that selenium both in inorganic and organic form passes the placental barrier of cats and rats (*Westfall et al.* 1938; *Rosenfeld* 1964). In those investigations the retention of selenium was higher in the fetal tissues after administration of organic selenium. The present investigation has demonstrated a high concentration of selenium in the placenta. The concentration of selenium in the fetus was much lower than in the mother. This indicates that the placenta constitutes a certain barrier for the passage of selenium from the mother to the fetus.

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SUMMARY

The distribution in the mouse of Se^{75} after injection of Se^{75}-sodium selenite was studied by autoradiography of sagittal whole-body sections. The animals were killed at various times, from 5 minutes to 8 days, after a single intravenous injection.

The distribution was characterized by a high blood level of selenium during the whole observation period.

The highest concentration of selenium soon after the injection was found in the liver, heart, kidney and adrenal cortex. Eight days after the injection the highest concentration was seen in the liver and the kidney. The selenium penetrated the placenta, but the uptake was much lower in the fetuses than in the maternal tissue.

ZUSAMMENFASSUNG

Die Verteilung von Selenium bei Mäusen auf Grund des Studiums mittels Autoradiographie des ganzen Körpers nach der Injektion von Se^{75}-Natriumselenit.

Die Verteilung von Se^{75} nach der Injektion von Se^{75}-Natriumselenit wurde bei der Maus mittels Autoradiographie sagittaler Körperschnitte studiert. Die Tiere wurden zu verschiedenen Zeitpunkten, von 5 Minuten bis zu 8 Tagen, nach der intravenösen Injektion getötet.

Die Verteilung kennzeichnete sich durch hohen Selengehalt im Blut während der ganzen Beobachtungszeit.

Die höchste Selenkonzentration fand sich gleich nach der Injektion in der Leber, im Herzen, in den Nieren und der Nebennierenrinde. Nach acht Tagen war der Selengehalt in der Leber und Niere am höchsten. Das Selen passierte die Plazenta, die Konzentration war jedoch in den Geweben des Fötus bedeutend niedriger als in denjenigen der Mutter.

SAMMANFATTNING

Distribution av selen hos mus studerad med helkroppsautoradiografi efter injektion av Se^{75}-natriumselenit.

Distributionen av Se^{75} efter injektion av Se^{75}-natriumselenit studerades hos mus med autoradiografi av sagittala kroppssnitt. Djuren avlivades vid olika tidpunkter, från 5 minuter till 8 dygn, efter den intravenösa injektionen.

Distributionen karakteriserades av hög selenhalt i blodet under hela observationstiden.

Högsta koncentrationen selen fanns strax efter injektionen i lever, hjärta, njurar och binjurebark. Efter 8 dygn var halten högst i lever och njure. Selenet passerade placentan, men koncentrationen var avsevärt lägre i fostrets än i moderns vävnad.

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