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RETENTION OF SELENIUM IN TISSUES OF SWINE AFTER A SINGLE INTRAMUSCULAR ADMINISTRATION OF SODIUM SELENITE*)

By

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The selenium retention in kidneys, liver, and skeletal muscle of pigs after prolonged feeding of therapeutic amounts of sodium selenite was recently studied (*Lindberg & Lannek* 1965). Small but significant increases of tissue selenium were noticed in the liver and skeletal muscle, but the kidney cortex showed no increase.

In the present investigation selenium was given intramuscularly at one occasion only (day 0). The pigs were killed at intervals during the next 14 days, and tissue samples were collected for analysis.

EXPERIMENTAL

Nine pigs, castrated males and females, of the Swedish Land Breed were used. From 1 week before selenium administration and onwards, they were given a commercial swine food (Forss "ungsvinsfoder"). All pigs were kept in the same pen until day 0. The control pigs were then placed in a separate pen in order to avoid selenium contamination. On day 0 the mean body weight was 94.2 kg (range 87.5—99.5 kg). Seven pigs were treated by an intramuscular injection of a preparation containing selenium (as sodium selenite) 0.6 mg per ml, and d'l-alpha-tocopherol acetate, 30 mg per ml ("Tocosef", Pharmacia). 0.1 ml was given

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per kg of body-weight. The injection was done at the side of the neck at a depth of 5 cm. The site of injection was exactly marked by tattooing. It had been confirmed previously on carcasses that the depot would be in the neck muscles, 1—2 cm from the subcutaneous fat layer. The pigs were killed by electrocution and bleeding at intervals shown in Table 1. A cube of muscle measuring 3—4 cm at the edge was taken from the neck below the tattooing mark. This was done in order to secure that very part of the muscle where the selenium deposition had taken place. The weights of the muscle cubes varied between 20 and 40 g. (In one pig, no. 3, the muscle sample only weighed 11 g). Skeletal muscle was also removed from the *M. gracilis*. Cortical tissue only was taken from the kidney. These and other tissue samples were stored separately in plastic bags at -20°C .

The *chemical procedure* has been described (*Lindberg & Sirén 1965*). The whole muscle sample from the site of injection was refluxed in concentrated HNO_3 until complete hydrolysis. It was then cooled, and fat residues were removed by filtration through glass wool. The filtrate was made up to 200 ml with concentrated HNO_3 . An aliquot was taken for determination of selenium.

The error of a single determination has been determined previously to be 4.44 % for kidney, 4.19 % for liver, and 2.38 % for muscle (*Lindberg & Lannek 1965*).

RESULTS AND DISCUSSION

The results are shown in Table 1. Increased selenium levels were observed at the site of injection, and in the liver, but not in the *M. gracilis* or in the kidneys.

The preparation used for injection contained selenium and alpha-tocopherol. These compounds are generally combined for prevention and therapy of selenium- and tocopherol-responsive animal diseases in this country. There is no evidence so far that alpha-tocopherol, when given at the same time as the selenium, will influence the distribution of the latter among different body tissues.

In most previous investigations on the distribution and elimination of selenium in animals either subtoxic amounts of the element, or radioselenium have been used. In the present study the treatment consisted of one single administration of a small selenium quantity, or approximately one fifteenth of the minimum lethal dose (*Orstadius 1961*).

Table 1. Selenium levels in pig tissues (ppm of dry substance) after a single intramuscular administration of selenium (as sodium selenite), 0.06 mg per kg body-weight, on day 0.

Pig no.	Killed on day	Kidney	Liver	M.gracilis	Site of injection
1*	0 (4 hrs.)	—	1.57	0.51	0.39
2	0 (4 hrs.)	11.9	4.47	0.54	0.81
3	3	10.1	2.18	0.49	0.45
4	3	10.3	2.38	0.55	0.79
5	7	11.0	1.54	0.50	0.75
6	7	10.6	1.57	0.52	0.44
7	14	10.1	1.36	0.48	1.23§
8	14	10.2	1.94	0.53	0.55
9*	14	9.6	1.32	0.54	0.40

* Non-treated controls.

— Missing value. In kidneys of comparable normal pigs fed the same commercial food as in the present study *Lindberg & Lannek* (1965) found 11.39 ± 1.10 (mean \pm standard deviation, $n = 7$).

§ Hematoma.

|| Abscess.

Following the intravenous injection of 1 mg of selenium (as sodium selenite) per kg of body-weight in rabbits, a rapid increase of the concentration in liver and kidneys was observed (*Smith et al.* 1938). Peak concentration was reached after 6 hours. Nearly half the injected amount was recovered from the urine within 48 hours. Only small amounts were found in the bile.

Similar results were obtained in rats after subcutaneous administration of sodium selenite containing radioselenium (*McConnell* 1941). They used higher doses than we have done, however, or 0.28—0.72 mg of selenium per rat.

In the dog a rapid elimination via the bile was observed under similar experimental conditions (*McConnell & Martin* 1952).

Buescher et al. (1961) dosed pigs orally with Se^{75} (as sodium selenite). It was shown that 71.5 % of the total excretion of Se^{75} was via the feces. The highest concentration in tissues after 120 hours was found in the kidney, and in the liver. They postulated that selenium may be a "high threshold substance" in the form of selenocystine or selenomethionine, as evidenced by its high concentration in the kidney, and low concentration in the urine.

Orstadius & Aberg (1961) studied the distribution of Se^{75} tagged selenium in normal pigs 7, 14, and 28 days after the administration. Significant radioactivity was observed at the injection site, and in the kidneys and the liver after 7 days and 14 days. They repeated the study in pigs with experimental muscular dystrophy and obtained similar results (*Ekman et al.* 1963).

Lindberg & Lannek (1965) observed no increase in kidney selenium in pigs whose food had been supplemented with 1.2 ppm of selenium (as sodium selenite) for 78 days. Similarly, the present study shows no elevation of kidney selenium. These observations are seemingly contradictory to earlier reports. Further experimental work will be necessary to answer these questions.

The increase in the liver of pigs no. 2, 3 and 4 which were killed after 4 hours, 3 days, and 3 days, respectively, probably reflect an excretory role of the organ. In pigs no. 5 and 6, killed after 7 days, the concentration was again on normal level. When selenium was given in the food for 78 days, the concentration in the liver did not reach higher values than after a single administration. From about 1 week and onwards the selenium levels remained at about 2.5 ppm, however. This may represent selenium which had been firmly bound in different tissues, and which was slowly released and eliminated. After a single administration the firmly bound selenium seems to be non-significant. The mode of elimination via the liver can be determined only by several determinations during the time when elimination proceeds.

The *M. gracilis* does not show any increased selenium content as compared with the control animals (pigs nos. 1 and 9, Table 1). The control pigs in the previous experiment (*Lindberg & Lannek* 1965) where the same commercial food was used also showed this level of concentration (0.52 ± 0.06 ppm of dry tissue). The neck muscle showed a lower content. It is true, that a larger piece of muscle was processed from the neck muscle than from the *M. gracilis*. This ought not have influenced the result. For control a large piece (a cube with 3 cm edge) of *M. gracilis* was analysed parallel with a small piece of the same muscle, both specimens being taken from the same pig and the same area of muscle. The selenium concentration of the large piece was 0.536 and 0.550 ppm, and of the small piece 0.56 and 0.53 ppm (double

determinations). The relatively low selenium concentration in the neck musculature may depend upon a higher fat content in the latter than in *M. gracilis*. This is indicated by the somewhat higher content of dry substance in the neck muscle (about 30 %) than in *M. gracilis* (about 25 %). The small pieces of *M. gracilis* were further trimmed free from visible fat. This was not done with the muscle cube from the neck, which was analysed *in toto*.

In pig no. 2 which was killed 4 hours after the treatment, the injection site showed 0.810 ppm of selenium. This is twice the concentration found in non-treated pigs. In pig no. 7, slaughtered on day 14, the concentration was still higher, or 1.23 ppm. The hematoma which was found here had obviously interfered with the resorption of the preparation.

The selenium concentrations at the site of injection otherwise decreased irregularly with time to a level close to that of non-treated pigs. There is one unexpectedly low value in pig no. 3, slaughtered on day 3. A small muscle sample had unfortunately been taken here. It is probable, that this small sample was not representative i. e. it may not have included that part, where the preparation was actually deposited.

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SUMMARY

The retention of selenium in pigs after a single intramuscular administration of a preparation containing sodium selenite and alpha-tocopherol was studied.

At the site of injection the concentration fell from 0.8 ppm to normal 0.4 ppm in about a week.

The concentration in the liver was 4.47 ppm after 4 hours, 2.18—2.38 ppm after 3 days, and on normal level (about 1.5 ppm) after 7 days. No increase was observed in the kidney or in the *M. gracilis*. It is concluded that the liver probably plays an important excretory role.

ZUSAMMENFASSUNG

Die Retention von Selen in Geweben des Schweines nach einer intramuskulären Injektion von Natriumselenit.

Die Retention von Selen bei Schweinen nach einer einmaligen intramuskulären Behandlung mit einem Präparat aus Natriumselenit und alpha-Tocopherol bestehend wurde untersucht. Die Konzentration am Injektionsplatz fiel von 0.8 p.p.m. zu normalen 0.4 p.p.m. während ungefähr einer Woche. In der Leber war die Konzentration 4.47 p.p.m. nach 4 Stunden, 2.18—2.38 p.p.m. nach 3 Tagen, und am normalen Niveau (ungefähr 1.5 p.p.m.) nach 7 Tagen. Die Verfasser ziehen den Schluss, dass die Leber eine wichtige Rolle als Ausscheidungsorgan spielt. In den Nieren und im *M. gracilis* wurde keine Erhöhung festgestellt.

SAMMANFATTNING

Retention av selen i vävnaderna av svin efter en intramuskulär injektion av natriumselenit.

Retentionen av selen hos grisar efter en intramuskulär administration av ett preparat innehållande natriumselenit och alfa-tokoferol har undersökts.

På injektionsplatsen föll koncentrationen från 0.8 ppm till normala 0.4 ppm under c:a 1 vecka.

Koncentrationen i levern var 4.47 ppm efter 4 timmar, 2.18—2.38 ppm efter 3 dygn, och på normal nivå (c:a 1.5 ppm) efter 7 dagar. Ingen stegring observerades i njurar eller *M. gracilis*. Författarna antar att levern spelar en viktig exkretorisk roll.

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