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RETENTION OF SELENIUM IN KIDNEYS, LIVER AND STRIATED MUSCLE AFTER PROLONGED FEEDING OF THERAPEUTIC AMOUNTS OF SODIUM SELENITE TO PIGS[1])

By

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It has been shown that a combination of sodium selenite and alfa-tocopherol protects pigs from experimental nutritional muscular degeneration (NMD) (8). The preparation[2]) has also proved to be effective for prevention and curative treatment of the spontaneous disease and is extensively used in the field.

With regard to possible harmful effects on human beings by selenium retained in the tissues of treated animals, the Swedish authorities have stated, that animals should not be processed for human consumption until 14 days have elapsed after the last treatment. This interval was suggested on the basis of studies on the distribution of Se^{75}-tagged sodium selenite in pigs affected with NMD (4). These authors believe that NMD pigs do not concentrate more selenium in muscles and organs than healthy pigs (7).

The retention of selenium in tissues of pigs, given sodium selenite perorally for varying periods, or intramuscularly, was studied by activation analysis (5).

We have determined the retention of selenium in some tissues of pigs, which were given sodium selenite as food additive for

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[2]) "Tokosel", Pharmacia.

78 days. The daily amount of selenium thus consumed corresponds to the dose, which is recommended for therapy as a single dose, or divided in two doses, given on consecutive days.

METHODS AND MATERIAL

Animals. Fourteen healthy pigs of the Swedish Land Breed, females and castrated males, were started on July 13 (day 0). They were divided in two groups (I and II) with 7 pigs in each. The mean body weight on day 0 was 19.6 kg (standard deviation 1.4 kg) in group I and 19.4 kg (standard deviation 3.1 kg) in group II. Both groups were fed a commercial food (Forss "Ungsvinsfoder") *ad lib.* Sodium selenite corresponding to 1.2 mg of selenium per kg (ppm) of food had been added and thoroughly mixed into the food given to group I. The mixing was done in a "Rapid" mixer, usually used for pharmaceutical drugs. The pigs had free access of water.

The two groups were kept in the same building but in different rooms in order to avoid selenium contamination of the food given to group II. Group I was given the selenium-supplemented food until day 78 and then the same unsupplemented basal food as group II.

The pigs were killed by electrocution and bleeding at intervals shown in Table 1. Tissue samples were immediately removed and stored separately in plastic bags at —20°C. Cortical tissue only was used from the kidneys. Striated muscle was removed from the M. gracilis.

The *chemical procedure* is described elsewhere (6). The fluorescence was determined by a Turner Fluorometer, model 111, using color spec. # 7—60 for peaks at 360 mμ as primary filter, and color spec. # 22, passing wave lenghts longer than 560 mμ, as secondary filter.

The error of a single determination (3) was computed from the double determinations shown in Table 1. The errors expressed as ppm of dry tissue (as per cent of mean value within brackets) are for kidney 0.493 (4.44), for liver 0.117 (4.19), and for muscle 0.016 (2.38), respectively.

RESULTS AND DISCUSSION

The results are shown in Table 1. The difference in selenium concentration between mean value of control food and selenium-supplemented food was, according to the analyses, 1.41 ppm. The

Table 1. Tissue selenium levels (ppm of dry substance) in supplemented group I and in control group II at intervals after interrupting selenium supplementation of food (1.2 ppm during 0—78 days). The selenium content of the control food was determined to be 0.126 ppm (mean value of 0.122 and 0.129 ppm at two determinations) and that of the selenium supplemented food 1.54 ppm (mean value of 1.42 and 1.65 ppm).

Killed on day	Kidney		Liver		Muscle	
	Groups					
	I	II	I	II	I	II
78[1])	11.6 ; 11.3	10.9	4.90 ; 4.50***	1.45	0.72 ; 0.80**	0.53
81	9.9 ; 9.9	11.5	1.81 ; 2.19 ; 1.85	1.87	0.75 ; 0.70*	0.61
81	10.4 ; 11.7	10.7	2.33 ; 2.25*	1.85	0.75 ; 0.69*	0.52
86	11.7 ; 11.5	13.2	2.50 ; 2.48*	1.98	0.67 ; 0.65	0.55
86	11.3 ; 12.4	9.7	3.07 ; 3.07***	1.98	0.68 ; 0.70*	0.42
92	10.4 ; 12.2	11.9	2.48 ; 2.60**	1.64	0.56 ; 0.61	0.54
92	11.2 ; 9.8	11.8	2.34 ; 2.60*	1.61	0.54 ; 0.58	0.48
mean		11.39		1.77		0.52
s. d.		1.10		0.20		0.06

*** mean value exceeding upper 99.9 per cent confidence limit of normal area.

** mean value exceeding upper 99.0 per cent confidence limit of normal area.

* mean value exceeding upper 95.0 per cent confidence limit of normal area.

[1]) the pigs were killed 4 hours after the last selenium-supplemented food was given to group I.

deviation from the value expected, i. e. 1.20 ppm, which was actually added, is probably caused by slightly heterogenous mixing of the sodium selenite. This is further supported by the fact that the difference between the two determinations of the control food is very small (0.003 ppm) in comparison with the determinations of the supplemented food (0.23 ppm). Anyhow, the analyses of the food support the assumption that the difference in selenium supply to the groups during 0—78 days was of the order of 1.2—1.4 ppm of the food and that these figures represent the actual selenium intake, provided that the food consumption of the two groups was equal. This seems to have been the case approximately. The mean body weight on day 74 was 67.1 kg (standard deviation 7.1) in group I and 72.1 kg (standard deviation 7.6) in group II. The difference between the mean values

is not significant ($P > 0.05$). It was observed when the animals were killed, that some of the pigs of group I had slight pneumonia of the enzootic pneumonia character. This was not present in group II. The two groups were not kept in the same room for reasons mentioned above. Apparently, the pigs of group I had accidently been contaminated. This may have caused some reduction of food intake and growth in group I. It does not seem to interfere with the conclusions to be drawn from the selenium retention figures, however.

It appears from Table 1 that significant retention of selenium in the supplemented group was observed in liver and muscle but not in kidneys. The most pronounced elevation is seen in the liver of the pig, which was killed 4 hours after the last selenium-supplemented meal. Even regarding this it is obvious that the continuous feeding of sodium selenite has not resulted in any noteworthy accumulation of selenium in the tissues examined. *Grant et al.* (5) added 0.2—0.4 ppm of selenium as sodium selenite to the food of pigs for varying periods and determined the retention in tissues by activation analyses. The selenium content of the basal food was not known, however, which makes a closer comparison with our results hazardous. They observed no symptoms of intoxication in their animals. Early investigations have shown that selenium does not appear to be accumulated (1). *Tucker* (12) concludes from toxicity experiments with sodium selenite that ewes, which were given ⅛ mg of Se/lb/day (approximately 0.275 mg/kg/day) over a period of two consecutive pregnancies, apparently could eliminate any excess of selenium. When the amount of selenium was doubled the sheep died after 9—28 doses.

Our results show that a considerable overdosing of sodium selenite, in comparison with therapeutically effective amounts, can be done in pigs with only small elevation of the selenium concentration in the tissues. The difference between the control animals and the dosed animals is of course also depending on the content of selenium in the basal food used to the controls. If this is deficient in selenium one should expect that the treated animals will store a relatively greater amount of the selenium given. If the basal food on the other hand permits the physiological stores to be filled, a smaller proportion of the added selenium will be retained. If large amounts of selenium are given the excretory mechanisms will be exhausted, significant

retention will take place and the animals may show symptoms of intoxication.

Human consumption of meat from animals which have been given the selenium doses used in this experiment apparently means a very small increase of selenium intake. Under therapeutic conditions this dose, as mentioned earlier, is given only once a month or so. Even the highest increase in selenium concentration noted, i. e. in the liver on day 78, means a level which is only half of what is normally observed in kidneys of non-treated pigs. Striated muscle is of special interest as being the main tissue used for human consumption. The highest values found here are of the order of 0.70—0.80 ppm. Investigations of meats for human food in seleniferous areas of North America showed selenium values between 1.17 and 8.00 ppm (10). It is further believed that a concentration of 5 ppm in common foods or one tenth this concentration in milk or water is potentially dangerous to man (9).

The observations in this investigation seem to infer that the legally stated 14 days period between a single peroral treatment with selenium at the level of 1.2 ppm of the food and the time of slaughter can be reduced. Three days is apparently sufficient for the liver to reach a level only slightly exceeding that of the non-treated animals. Normally, only sporadic animals treated curatively or prophylactically against NMD are slaughtered until a long period, i. e. several weeks or more, has elapsed.

Treatment by intramuscular injection involves a factor, which is not dealt with in this investigation, namely the retention at the place of injection. This problem will be discussed in a paper to be published. The same applies to the retention in tissues other than kidneys, liver, and striated muscle after peroral and intramuscular administration of selenium.

The peak concentration in the liver of the pig killed 4 hours after eating the last meal of selenium-supplemented food may indicate that the liver holds a main position as an excretory organ under the conditions of this experiment. *Cousins et al.* (2) found in sheep that faecal excretion was invariably higher than the amount excreted in the urine. They suggest either non-absorption of ingested selenium or intestinal excretion as an explanation.

We find it difficult at the moment to explain the abscence of selenium retention in the kidneys, as these have mostly been accepted as main excretory organs (9). Most earlier experiments

have been done either using large doses of selenium or radioselenium. It is possible that a marked overloading leads to the use of many excretory channels, some of which do not take part in the normal, slow elimination.

Radioselenium studies obviously give information on the rate of turnover. The observation of a high rate of turnover in an organ like the kidneys, which are certainly active in many kinds of excreting processes, does not necessarily implicate excretory activity with regard to selenium.

Experiments using selenium in amounts, comparable with ours, in sheep (2) show without doubt, however, that selenium was eliminated with the urine. Species differences are possible and further information ought to be collected concerning the pigs on this point.

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SUMMARY

Sodium selenite was mixed in a commercial pig food so as to increase the original selenium content (0.126 ppm) by 1.2 ppm. The supplemented food was given to 7 pigs during 78 days. A control group of as many pigs was given the non-supplemented commercial food. The pigs were killed, starting 4 hours after the last selenium-supplemented food had been given and then with intervals during the next 14 days. Small but significant increases of tissue selenium were noticed in liver and striated muscle as compared with the control animals. The kidneys showed no increase.

ZUSAMMENFASSUNG

Über die Zurückhaltung von Selen in Niere, Leber und querstreifiger Muskulatur des Schweines nach langzeitiger Verabreichung therapeutischer Gaben von Natriumselenit.

Natriumselenit wurde einem kommerziellen Schweinefutter beigemengt so dass das Gehalt (0.126 ppm) mit 1.2 ppm erhöht wurde. Das supplierte Futter wurde 7 Schweinen während 78 Tagen gegeben. Das unsupplierte kommerzielle Futter wurde einer Kontrollgruppe derselben Anzahl von Schweinen gegeben.

Die Schweine wurden getötet mit Beginn 4 Stunden nach dem letzten Füttern von selensuppliertem Futter und dann mit Intervallen während der nächsten 14 Tagen. Kleine aber signifikante Erhöhungen des Selens im Vergleich mit den Kontrollen wurden in der Leber und in querstreifiger Muskulatur gefunden. Die Nieren zeigten keine Erhöhungen.

SAMMANFATTNING

Retention av selen i njurar, lever och tvärstrimmig muskulatur hos svin efter långvarig utfodring med terapeutiska doser natriumselenit.

Natriumselenit inblandades i ett kommersiellt svinfoder, så att den ursprungliga selenhalten (0.126 ppm) ökades med 1.2 ppm. Det supplementerade fodret gavs åt 7 griser under 78 dagar. En kontrollgrupp på lika många djur gavs det osupplementerade kommersiella fodret.

Grisarna dödades med början 4 timmar efter den sista utfodringen med selenberikat foder och därefter med intervaller under de följande 14 dagarna. Små men signifikanta stegringar av vävnadsselen observerades i lever och tvärstrimmig muskulatur. Njurarna visade ingen stegring.

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