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FLUOROMETRIC SELENIUM DETERMINATIONS IN THE LIVER OF NORMAL PIGS AND IN PIGS AFFECTED WITH NUTRITIONAL MUSCULAR DYSTROPHY AND LIVER DYSTROPHY*)

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It was shown in a preliminary report (7) that kidneys of pigs affected with nutritional muscular dystrophy (NMD) or liver dystrophy (LD, hepatosis diaetetica) have reduced selenium concentration. The kidneys are of special interest since they are probably the organ most rich in selenium (3, 4, 11). The liver may come next in order of selenium concentration. Unlike in other domestic animals but similar to rats and mice (13) the liver of pigs manifests dystrophy under supposed conditions of deficiency in vitamin E and selenium (5, 8).

MATERIAL AND METHODS

Part of the *animal material* has been described elsewhere (1). Three healthy control pigs (E/4, 5, 6), one pig affected with NMD (M/1), and one with LD (N/1) have been added. Pigs E/4 and N/1 weighed about 10 kg each. Pigs E/5 and E/6 were 1 day old, weighing about 1.5 kg each. Pig M/1 weighed about 40 kg. Pigs E/4, 5, 6 and M/1 were bled to death under ether narcosis. Pig N/1 died spontaneously.

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The diagnosis was established post mortem macro- and microscopically, and living pigs, which were ultimately killed, also by serum enzyme tests (10).

The pigs belonged to the Swedish Land Breed (lantras).

Method for selenium determination. Tissue samples, weighing about 500 mg, were wet ashed in 100 ml flasks with *Bethge*'s apparatus (1) using 5 ml of 70 % perchloric acid + 10 ml of 65 % nitric acid. Two ml of H_2O were added and residues of nitric acid evaporated. After adding 10 ml of H_2O, the solution was neutralized with 50 % KOH. Precipitated $KClO_4$ was removed by filtration through glass wool and washed with 2 × 5 ml of H_2O. The eluate was buffered with 3 ml of 25 % potassium citrate, pH 5.6. Two ml of 1 % diethyldithiocarbamate solution was added (2). The diethyldithiocarbamate selenium complex was extracted with 3 × 15 ml of petroleum ether (b.p. 30—60°C). The solvent was evaporated in water bath and the residue was wet ashed with 1 ml of perchloric acid + 5 drops of nitric acid. One ml of H_2O was added, and residues of nitric acid was removed by evaporation. After addition of 10 ml of H_2O the solution was neutralized with 50 % KOH. Precipitated $KClO_4$ was removed by filtration and washed. The eluate volume was adjusted to 45 ml by addition of water, and 5 ml of 1 N HCl was added. The samples were taken to 75 ml test tubes, which were placed in water bath at 50°C. Five ml of 0.01 % 2,3-diaminonaphtalein in 0.1 N HCl were added (12). The developed 4,5-benzopiazselenol was extracted with 5 ml of cyclohexane (spectral grade). The extract was washed with 2 × 35 ml of 0.1 N HCl and centrifuged. The fluorescence was determined by a Zeiss spectrophotometer PMQ II with fluorometer equipment at 560 mμ and filter E as the primary filter.

In each series a reagent blank and 2 standard samples (0.2 and 0.4 μg of Se, respectively) were included. All samples contained 0.2 ml of Se^{75} solution, initially corresponding to 10.000 cpm/ml. This was done in order to check losses during the analytical procedure. After the determination of fluorescence the radioactivity of each sample was measured in a well crystal scintillator.

RESULTS AND DISCUSSION

The recovery of selenium as determined by measuring radioactivity is about 70 %. Comparison between radioactivity of test sample and of standard gives 88—105 %. The results of selenium determination in livers are shown in Table 1. Most of the kidney values, which are included in the table, have been published elsewhere (7). Some have been added, however, and it was found convenient to have all kidney values included in the table.

The grouping of normal animals according to body weight (or age) gives the impression of a positive correlation between body-weights and kidney-selenium. Thus, all pigs of slaughter

Table 1. Selenium determinations in pigs kidneys and livers. Results in parts per million of dry weight.

Normal				Muscular dystrophy				Liver dystrophy			
Farm/pig number	Kidney	Liver		Farm/pig number	Kidney	Liver		Farm/pig number	Kidney	Liver	
A/1	9.80+	0.89		F/1	3.94	0.24		I/1	2.62+	0.21	
/2	9.54+	1.00		/2	4.66+	0.22		/2	3.82+	0.24	
/3	13.45	1.10		/3	3.04	0.13		K/1	2.18	0.13	
B/1	12.20	0.79		G/1	3.20+	0.28		N/1	2.18	0.13	
/2	12.55	1.09		/2	3.21+	0.22					
C/1	11.80	1.72		H/1	2.54	0.16					
/2	10.40	1.56		M/1	1.38	0.21					
D/1	13.40	1.74									
/2	13.15	1.07									
E/1	8.53+	1.14	; 1.20								
/2	8.60	1.09	; 1.17								
/3	8.69	0.89	; 0.95								
/4	5.80	2.12	; 2.12								
/5	4.80	1.63	; 1.45								
/6	3.60	0.59	; 0.52								
K/2	4.66	1.20									
Mean ± error of mean		1.23 ± 0.42		Mean ± error of mean		0.21±0.05				0.18±0.05	
				Significance of difference versus normal		P<0.001				P<0.001	

Error of single determination = 0.10 ppm or 8.0 % of the mean value of all double determinations.

+ = Mean of double determinations.

weight, although originating from different herds, have 9 ppm of Se or more in their kidneys. Smaller pigs have lower levels, two new-born ones having 4.80 and 3.60 ppm, respectively. Most small pigs came from one herd (E), however, and this makes reliable conclusions difficult to draw. The relatively low kidney values of the E pigs may be characteristic of the herd and not of the age. Additional material, which is not included here, supports the assumption, however, that young pigs have lower selenium concentration in the kidneys than bigger ones.

The livers of the normal pigs do not show any corresponding variation. The selenium values vary roughly between 0.5 and 2 ppm irrespective of the age of the animals. The livers of pigs affected with NMD or LD are significantly poorer in selenium.

It is difficult to establish a "normal" tissue level of selenium. Some animals may have obtained more Se than they actually need and for that reason show high levels. Other animals may be in a slightly deficient condition but not show any manifest disease as yet. This is apparently the case with pig K/2, which had a low kidney value, but a liver value which corresponds to the control group. The pig showed no symptoms of disease and was normal at post mortem examination. It came from a litter, however, where one pig (K/1) had developed LD and died. The latter had a low selenium level in the kidney as well as in the liver. The low kidney selenium concentration in pig K/2 may therefore actually indicate a state of deficiency, and the pig might have fallen ill, if it had been left alive and untreated. It may be inferred that, within certain limits, the liver concentration is kept at a more constant level irrespective of the supply. A final decrease below a critical borderline would be associated with NMD or LD. According to our figures this borderline should lie between 0.5 and 0.25 ppm. Actually, we have obtained liver selenium values as low as 0.36 ppm in new-born piglets with no indications of MD or LD. Several weeks later their littermates fell ill with MD, however.

Grant et al. (6) determined selenium in tissues of healthy pigs by activation analysis. Our values correspond fairly well to those, which they obtained after chemical separation.

The low values of tissues in pigs affected with MD or LD are in good agreement with the favourable therapeutic results obtained by administration of selenium (9).

Our figures do not indicate any differences in liver selenium

concentration between MD and LD cases. The number af animals is small, however, and the existence of a slight difference cannot be excluded.

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SUMMARY

The selenium concentration of livers and kidneys in normal pigs and in pigs affected with nutritional muscular dystrophy (NMD) and liver dystrophy (LD) was determined. The kidney values showed great variation in normal pigs. The young pigs generally had the lowest levels. The liver values were less scattered.

In NMD and LD the liver contained about 0.2 ppm of selenium on the average, whereas normal livers contained about 1.2 ppm. Kidneys showed corresponding differences. This is in good agreement with the favourable results obtained with selenium therapy.

ZUSAMMENFASSUNG

Fluorometrische Selenbestimmungen in der Leber normaler Schweinen und Schweinen von ernährungsbedingter Muskeldystrophie angegriffen.

Die Konzentration von Selen in der Leber und in den Nieren bei normalen Schweinen und bei Schweinen die von ernährungsbedingter Muskeldystrophie (NMD) und Leberdystrophie (LD) angegriffen waren, wurde bestimmt. Das Gehalt der Nieren zeigte grosse Variation bei normalen Schweinen. Die jungen Schweinen hatten das niedrigste Gehalt. Das Gehalt der Leber zeigte kleinere Variation. Bei NMD und LD war das Gehalt von Selen der Leber ungefähr 0,2 ppm, das der normalen Leber ungefähr 1,2 ppm., In den Nieren fand man eine ähnliche Differenz. Diese Observationen sind in guter Übereinstimmung mit den guten Resultaten der Selenbehandlung gegen die beiden Krankheiten.

SAMMANFATTNING

Fluorometriska selenbestämningar i levern hos normala svin och hos svin angripna av nutritionell muskeldystrofi och leverdystrofi.

Selenhalten i lever och njurar hos normala grisar samt hos grisar med nutritionell muskeldystrofi (NMD) och leverdystrofi (LD) bestämdes. Njurvärdena visar ganska stor variation hos normala grisar. De minsta grisarna har de lägsta värdena. I levern finns inte någon motsvarande variation. Vid NMD och LD visar levern ungefär 0.2 ppm i medeltal medan normala levrar innehåller ungefär 1.2 ppm. I njurarna fanns motsvarande differenser. Observationerna står i god överensstämmelse med de gynnsamma resultaten av selenbehandlingen vid de nämnda sjukdomarna.

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