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Selenium Deficiency and Protein, RNA and DNA Synthesis in Rat Pancreas and Liver (38390)

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Selenium is associated with proteins in both procaryotes and eucaryotes (1). In the growth of *E. coli* in a medium containing $^{75}SeO_3$, selenium is incorporated into tRNA bases (2), where it is present in at least one of the minor bases as 4-selenouridine (3). In a cell-free system selenomethionyl tRNA participates in polypeptide synthesis to about the same extent as does methionyl tRNA (4). A parallelism exists between sulfur (Cyst and Met) and seleno (Se Cyst and Se Met) amino acid metabolism in protein synthesis in the skin and other tissues of the Zn-deficient rat (5). Since selenium is associated with (a) protein, (b) RNA and (c) indirectly, DNA, experiments were undertaken to study protein, RNA and DNA metabolism in the selenium-deficient (Se (D)) rat, specifically in the pancreas because it has been found that a definite depression occurs in specific enzymes in the pancreas of (Se D) chicks) (6).

Methods and Procedures. Weanling male rats of Sprague–Dawley strain were kept on a torula yeast diet. The basal diet was composed of 30% torula yeast[1], 59% sucrose[2], 5% salts (7), 5% stripped lard[3], and 1% vitamin mixture[4], providing Vitamin E as *d*-α-tocopherol (100 mg/kg). The diet of one group of rats was supplemented with 0.5 ppm Se as sodium selenite. The rats were fed *ad lib.*, and at the end of the experiment, those on the selenium-supplemented diet were slightly heavier than the ones on the selenium-deficient diet. After 66 days on the diets and after overnight fasting and 2 hr prior to decapitation, one group of rats received intramuscularly glycine-2-^{14}C (20 μCi/100 g body wt) and uridine-5, 6-^{3}H (50 μCi/100 g body wt) and another group of rats received thymidine-^{3}H (30 μCi/100 g body wt). Protein, RNA and DNA were isolated and assayed for radioactivity. The method described by Wannamacher *et al.* (8) was used for the determination of DNA, RNA and radioactivity. In the preparation of protein fractions from pancreas and liver, the tissue was homogenized in a Potter–Elvehjem homogenizer and processed according to methods already described (9). Tissue protein concentrations were estimated by the method of Lowry *et al.* (10) with bovine serum albumin as standard. Samples for radioactive assay were dissolved in Hyamine solubilizer and counted in a Packard Counter Model 3320. Stable selenium was determined by neutron activation analysis (11).

Results and Discussion. Stable selenium content of tissues from Se (D) and selenium-supplemented (Se (S)) rats after 33 and 66 days on the diets is shown in Fig. 1. The difference between the Se (S) and Se (D) rats was statistically significant, $P < 0.01$, in all the tissues examined. Liver at the 33-day time interval and muscle at 66 days were void of selenium. As shown in Table I there was a significantly smaller weight gain in the Se (D) rats than in the Se (S) ($P < 0.01$). The radioactivity values (Sp. A.) for protein RNA and DNA in selenium-deficient rat pancreas and liver are shown in Table I and Expt 1 and 2. The ^{14}C content from glycine in both the pancreatic (Table I, Expt 1) and liver (Table I, Expt 2) proteins was not statistically different in the Se (D) and the Se (S) rats. Further, there was

[1] Lake States Yeast Corp., Rhinelander, WI.

[2] Commercial, regular grade.

[3] Vitamin E-free animal fat, stripped by molecular distillation (Distillation Products, Inc., Rochester, NY).

[4] Lactose 97.861 g, thiamine HCl 40 mg, riboflavin 25 mg; pyridoxine HCl 20 mg; DL-calcium panthothenate 44 mg; choline chloride 1 g; niacin 1 g; menadione (2-methyl-1, 4-napthoquinone) 10 mg.

TABLE I. Experiment I. Protein, RNA, and DNA in Selenium Deficient Rat Pancreas.[a]

Type of Diet	Body Wt. g	Protein Level mg protein/g tissue	Protein Sp. act.[b] DPM/mg protein × 10^3	RNA Level mg RNA/g tissue	RNA Sp. act.[b] DPM/mg RNA × 10^3	DNA Level mg DNA/g tissue	DNA Sp. act.[c] DPM/mg DNA × [3]
Se-deficient	260 ± 17[e]	119 ± 8.9	7.77 ± 0.810	17.0 ± 2.2	31.1 ± 4.60	1.92 ± 0.23	12.84 ± 2.24
Selenium supplemented[d]	280 ± 15	120 ± 19.8	8.04 ± 1.81	16.7 ± 1.8	28.36 ± 4.86	1.81 ± 0.19	12.45 ± 2.43
Experiment II: Protein, RNA, and DNA in Selenium Deficient Rat Liver.[a]							
Se-deficient		131.5 ± 5.9	5.13 ± 0.61	9.66 ± 0.65	20.41 ± 2.95	1.62 ± 0.34	35.86 ± 11.41
Selenium-supplemented[d]		146.5 ± 19.6	5.02 ± 1.38	8.99 ± 1.09	22.49 ± 2.37	1.69 ± 0.05	28.64 ± 2.13

[a] 18 weanling male rats of Sprague–Dawley strain were kept on a torula yeast diet for 66 days. Twelve were supplemented with Vitamin E, and six were on the same diet supplemented with selenium which acted as controls.

[b] Two hours prior to decapitation six Se-deficient rats received intramuscularly glycine-2-^{14}C [Sp. act.: 5 Ci/m*M* (20 μCi/100 g body wt)] and Uridine −5, 6-^{3}H [Sp. act. :42 Ci/m*M* (50 μCi/100 g body wt)].

[c] Two hours prior to decapitation 6 Se-deficient rats received thymidine [methyl-^{3}H]. [Sp. act. 6.7 Ci/mm (30 Ci/100 g body wt)].

[d] *d*—α tocopherol acetate, 100 mg/kg, added to diet of both groups.

[e] Mean ± SD ($P < 0.01$).

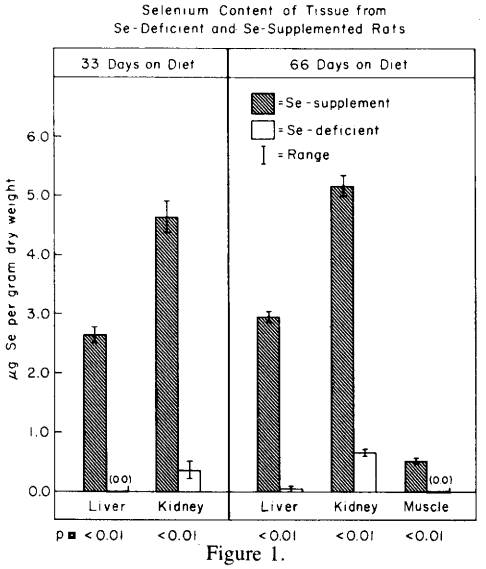

Figure 1.

no detectable difference in the incorporation of uridine-^{3}H and thymidine-^{3}H into RNA and DNA respectively, in the pancreas (Table I, Expt 1) and liver (Table I, Expt 2) of the Se (D) rats as compared to the Se (S) rats. Our observations indicate that in the selenium-deficient rat there appears to be no functional change, within the conditions of our experiments, of the pancreas and liver protein, RNA and DNA metabolism. Thus, the pancreatic enzymic impairment observed by Scott *et al.* due to selenium deficiency in the chick (6), does not occur in the rat. Regardless of low selenium levels in tissues of selenium-deficient rats with Vitamin E supplements, as shown in Fig. 1, protein RNA and DNA synthesis is not significantly different from the selenium plus Vitamin E supplemented rats. It is known that Vitamin E and selenium spare each other nutritionally under some conditions, for example in the prevention of exudative diathesis in chicks (12, 13). Usually there is an increased need for Vitamin E as the level of selenium is reduced, until the point at which no further addition of the vitamin suffices. It may be that the nutritional need for selenium by rats in the presence of ample Vitamin E (100 mg/kg) was so small that it was met by selenium contamination of the basal diet. However, it is certain that the need for selenium cannot be completely eliminated by Vitamin E. On the other hand, rats given the basal diet devoid of both selenium and Vitamin E died from liver hemorrhage in 21 days, attesting to the severity of the selenium deficiency. Our results suggest that apparently Vitamin E and selenium act independently in the pancreas and liver. This is in contrast to Diplock's hypothesis that these nutrients interact in some aspects of membrane function (14).

Summary. Rats on a selenium-deficient torula yeast diet containing 100 mg Vitamin E/kg compared to a similar diet with 0.5 ppm selenium as sodium selenite do not show any decrease in protein, RNA and DNA synthesis in the pancreas or liver. The gross depression and change of pancreatic enzymes found in the chick on selenium-deficient diet were not apparent in the rat on a selenium-deficient diet.

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