Lack of Effect of Selenium on Glycolytic and Citric Acid Cycle Intermediates in Rat Kidney and Liver[1] (37663)

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Selenium has been classified as an essential trace element for a variety of mammals and birds (1), and recent evidence indicates that one of its biochemical functions involves glutathione peroxidase (2). However, ingestion of increased amounts of selenium by experimental animals produces toxicity, such as decreased food consumption (3). On the other hand, three independent epidemiological studies among children in four western states of the United States, reviewed elsewhere (4, 5), and also a recent study in three population groups in the Soviet Union (6) have shown that consumption of somewhat increased amounts of dietary selenium during the period of tooth development increases the prevalence of dental caries. Experiments with rats (7) and monkeys (8) corroborate the above findings in humans. We recently demonstrated that most of the ^{75}Se incorporated into enamel and dentine during tooth development is present in the protein fraction of these two dental tissues (9), where it is believed to inhibit tooth mineralization (10, 11).

The underlying biochemical mechanism of selenium toxicity in the intact animal has not been explained, although there is evidence that many enzymes which require sulfhydryl groups for activity are inhibited *in vitro* by selenium (1, 3). In this regard selenium has also been shown to be associated with a variety of tissue proteins as selenocystine and selenomethionine (12). Numerous metabolic changes occur in animals receiving increased amounts of selenium (1, 3), but there has been no systematic search along metabolic pathways in the intact animal to discover if enzyme inhibition is the underlying mechanism of selenium toxicity.

Thus the purposes of this study were: 1) to determine if dietary selenium causes changes in the concentrations of glycolytic or citric acid cycle intermediates, which could be used to infer points of enzyme inhibition by selenium in the tissues of the intact animal, and 2) to develop a suitable feeding apparatus which would eliminate the effects of depressed food consumption on the metabolic studies.

Methods and Experimental Plan. Eighteen weanling male Holtzman rats (Madison, Wisconsin) were individually caged in raised-screen bottom cages and equally divided into 3 groups of 6 animals each. The animals in each group received drinking solutions and pelleted Purina Laboratory Chow (97 mg pellets, 5% acacia and 1.25% glycerin binding agents; P. J. Noyes Company, New Hampshire) in the following manner: the *ad libitum* control group, distilled drinking water and pelleted diet; the selenium group, distilled drinking water containing 4 ppm Se (as Na_2SeO_3) and pelleted diet both *ad libitum*; and the yoke control group distilled drinking water *ad libitum* and pelleted diet dispensed to the rats by a yoke feeding apparatus.

The purpose of the yoke feeding apparatus was to force the yoke control rats to consume diet in the decreased amounts and altered pattern of food intake of the selenium rats. One control rat was yoke-fed to one selenium rat; thus there were six pairs of animals in the yoke feeding apparatus. The selenium rats consumed pelleted diet from programmed food cups (Behavioral Controls Inc., Milwaukee, Wisconsin) which are stain-

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TABLE I. Effect of Selenium and Yoke Feeding on Body Weights.

	Ad libitum control	Selenium (4 ppm)	Yoke control
Initial body weight	44 ± 1[a]	46 ± 0	46 ± 0
Final body weight (22 days)	182 ± 6[b]	141 ± 3[c]	152 ± 4[c]
Body weight gain	161 ± 5[b]	96 ± 3[c]	107 ± 4[c]

[a] Mean ± standard error. There were 6 animals per group. Results in g.
[b,c] Means within the same horizontal column having different superscripts are significantly different, $p < 0.005$; means within the same horizontal column having the same superscripts are not significantly different, $p > 0.05$.

less steel vertical food cups each containing a hinged baffle connected to a microswitch. Each time a selenium rat removed a pellet from his food cup, he pushed the baffle and tripped the microswitch. This signaled a pellet dispensing mechanism (Behavioral Controls Inc.) to deliver a pellet of diet to the food cup of the yoke control rat and a replacement pellet of diet to the programmed food cup of the selenium rat. The pellet hopper of the pellet dispensing device was replenished daily with a 24-hr supply of pellets, which were dispensed by the device to the food cups via latex surgical tubing.

After an experimental period of 22 days, the rats were killed by cervical fracture, and the livers and kidneys were rapidly removed (less than 10 sec) and frozen in liquid nitrogen. In order to study the effect of dietary selenium on glycolysis and the citric acid cycle, 9 glycolytic and 3 citric acid cycle intermediates were measured in neutralized perchloric acid extracts of the livers and kidneys by enzymatic methods previously described (13, 14). Liver and kidney tissues were studied because they have been shown to accumulate high concentrations of selenium (3) and, therefore, presumably liable to selenium enzyme inhibition.

Results and Discussion. The administration of drinking water containing 4 ppm Se to weanling rats for 22 days caused a 23% reduction in final body weights and a 41% reduction in body weight gains of the selenium rats compared to the *ad libitum* control rats (Table I). The control animals which were yoke-fed to the selenium animals consumed each pellet of diet as soon as it was delivered to the food cup since they were essentially hungry normal rats forced to consume less than normal amounts of diet according to the decreased appetite of the selenium rats. The selenium rats were observed to readily enter the programmed food cup and activate the pellet dispensing device, but they sometimes either did not take the pellet or they dropped it through the bottom of the cage. Despite this difficulty, the final body weight and the body weight gain of the yoke control rats were not statistically different from those of the selenium rats (Table I). The yoke feeding apparatus thus produced animals which were a much more suitable control group for the metabolic studies presented below than were the *ad libitum* control animals, whose eating habits were different from the selenium and yoked control animals. Therefore, metabolite data are presented below only for the selenium and the yoke control groups.

There were no statistically significant changes in the concentrations of any of the glycolytic or citric acid cycle intermediates in either the liver or the kidneys of the selenium rats compared to the concentrations of intermediates in the yoke control rats[2] (Table II). Previous studies on chronic selenosis in animals have noted changes in numerous metabolites including blood glucose and liver glycogen (1, 3). However, because of the lack of a proper control group, it was usually impossible to discern if these changes were due to an indirect effect of decreased food consumption. The results of the present experiment, in which the control rats were

[2] Abbreviations used are: G-6-*P*, glucose-6-phosphate; F-6-*P*, fructose-6-phosphate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; PEP, phosphoenol-pyruvate; α-KG, α-ketoglutarate; ATP, adenosine-5′-triphosphoric acid.

TABLE II. Effect of Dietary Selenium on the Concentrations of Liver and Kidney Glycolytic and Citric Acid Cycle Intermediates.

	Liver		Kidney	
Intermediates	Yoke control	Selenium (4 ppm)	Yoke control	Selenium (4 ppm)
Glucose	3.228 ± 0.440[a]	3.748 ± 0.644	3.485 ± 0.222	3.183 ± 0.136
G-6-*P*	0.251 ± 0.034	0.161 ± 0.028	0.018 ± 0.009	0.012 ± 0.008
F-6-*P*	0.043 ± 0.011	0.017 ± 0.008	0.003 ± 0.002	0.001 ± 0.001
3-PGA	0.226 ± 0.028	0.224 ± 0.030	0.609 ± 0.154	0.545 ± 0.127
2-PGA	0.056 ± 0.011	0.072 ± 0.020	0.247 ± 0.078	0.223 ± 0.057
PEP	0.078 ± 0.009	0.098 ± 0.017	0.201 ± 0.082	0.225 ± 0.059
Pyruvate	0.051 ± 0.008	0.069 ± 0.015	0.153 ± 0.061	0.207 ± 0.067
Lactate	0.624 ± 0.169	0.527 ± 0.107	0.989 ± 0.095	0.967 ± 0.190
Citrate	0.341 ± 0.071	0.250 ± 0.045	0.863 ± 0.126	0.854 ± 0.150
α-KG	0.082 ± 0.007	0.109 ± 0.023	0.442 ± 0.053	0.417 ± 0.045
Malate	0.254 ± 0.030	0.232 ± 0.032	0.584 ± 0.022	0.598 ± 0.025
ATP	1.321 ± 0.159	1.349 ± 0.203	0.940 ± 0.058	0.991 ± 0.076

[a] Mean ± standard error for 6 observations per mean, in μmoles/g, wet.

yoke-fed to the selenium rats, indicate that selenium does not cause inhibition of enzymes within glycolysis or the citric acid cycle, and that metabolic lesions within these two pathways were not the cause of the significant growth depression noted in the present experiment.

Besides decreasing the total amount of food and water intake, dietary selenium has been shown to alter the physiological ratio of food to water intake and to alter many of the parameters associated with the normal eating and drinking behavior of the rat (15–17). In view of the results of the present study, changes in the feeding and drinking behavior in animals receiving increased amounts of selenium may be due to an unknown effect of selenium on metabolism outside of glycolysis and the citric acid cycle. The offensive odor and/or taste of selenium added to the water or the diet of the animals is also believed to be an important contributory factor. The odor of selenium in drinking water was easily detected by the author (T. R.S.) at the 4 ppm level used in the present experiment.

Summary. The concentrations of glycolytic and citric acid cycle intermediates were measured in the livers and kidneys from a selenium group of rats receiving 4 ppm Se in their drinking water. Compared to the concentrations of intermediates in the livers and kidneys from a control group of rats yoke-fed to the selenium rats, no metabolic changes were observed. It was concluded that growth depression and changes in eating and drinking behavior caused by selenium were not due to enzyme inhibition within glycolysis or the citric acid cycle.

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