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Adaptation of Rats to Selenium Intake

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ABSTRACT Changes of liver selenium after intake of organic selenium at different levels and for various lengths of time were studied in 236 young rats. In litters born to mothers fed a diet containing 4.5 ppm selenium (in seleniferous sesame press cake) and kept on this diet after weaning, the liver levels decreased steadily; these levels increased in stock rats fed the same diet. The graphical presentation of these changes resulted in two straight lines, crossing each other. When the selenium diet was fed to dams 5 to 7 days prior to parturition and thereafter, the liver levels of the litters rose rapidly; liver levels increased at a slower rate in rats born to females fed the diet 15 to 19 days prior to birth and decreased in litters from mothers kept on the diet prior to mating. When litters from stock females were nursed by dams fed the seleniferous diet during pregnancy and changed to the stock ration after the birth of their litters, the liver selenium levels rose rapidly for about 3 weeks after weaning and then decreased. On a diet containing 10 ppm selenium, liver values in rats from the stock colony rose for 2 to 3 weeks to high levels and then decreased. Animals bred on the diet containing 4.5 ppm selenium exhibited a slower, more continuous rise of liver selenium when the high selenium diet was fed. The results suggest the existence of an adaptation mechanism which allows rats exposed to chronic selenium ingestion to store less of this element than previously unexposed controls.

In previous experiments on the effect of selenium in growing rats, it was observed that a dietary level of this element which resulted in a small but significant growth depression in young animals from the stock colony was without notable effect in litters born to dams which had been kept on an experimental seleniferous diet prior to mating (1). This was quite unexpected because higher selenium body levels at the start of the experiment would have made the opposite result more likely, and also because young animals are often more severely affected by dietary injury when exposed to it during embryonic life. In another study from this laboratory, it was found that the characteristic drop in hematocrit and hemoglobin levels in rats fed high selenium diets was significantly delayed when the experimental animals had been bred on a seleniferous ration as compared with controls bred on stock rations, (2). These observations pointed to the possible existence of some adaptation to chronic selenium intake in rats. The present experiments were undertaken to investigate whether such a mechanism could be detected through the accumulation of selenium in the liver.

MATERIAL AND METHODS

The experiments were performed with 236 rats descended from the Sprague-Dawley strain, bred in this Institute for about 20 years. They were kept in individual screen-bottom cages. Food and water were offered ad libitum, and the stock diet was a commercial laboratory ration.[1] Each bag was analyzed for selenium content and only those lots with values below 1.0 ppm were used.

Two lots of expeller processed sesame press cake with a selenium content of 0.8 and 23.3 ppm, respectively, were used. They were ground in a hammer mill. By mixing appropriate portions of these lots, diets containing 4.5 and 10 ppm selenium, respectively, were prepared. They contained 16.4% protein. The rest of the diet consisted of the following components: (in percent) corn oil with vitamins A and D added, 5; salt mixture USP XIV, 4; L-lysine·HCl, 0.4; vitamin B mixture (3), 1; and cassava starch to make up 100%.

Animals of experiments 1 and 4 had been weaned at 28 days, those of experiments 2 and 3 at 22 days. They were killed at convenient periods by a blow on the head; the livers were extirpated, weighed, dried at 100° to constant weight, ground in a laboratory mill [2] and duplicate samples of about 100 mg burned in oxygen in a

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[1] Ralston Purina Company, St. Louis, Mo.
[2] Wiley, Intermediate Model, Arthur H. Thomas Company, Philadelphia, Pa.

2-liter Schöninger flask containing 20 ml of bidistilled water. After cooling the content was stirred with a magnetic stirrer for 10 minutes and selenium was determined in a filtered aliquot by the fluorometric method of Dye et al. (4) with 3,3-diaminobenzidine tetrahydrochloride. Two to five animals were killed in each case and the mean liver selenium values were calculated on the fresh weight basis.

Five different experiments were performed. In experiment 1, the selenium contents of the livers of two groups of rats fed a diet containing 4.5 ppm selenium were compared: one group of 34 animals had been bred and nursed on the stock ration and was fed the selenium diet since weaning; the mothers of the second group of 37 animals had been fed the selenium diet 5 days prior to mating and the litters were continued on the same diet after weaning. For mating, one male was kept for 5 days in the cage of one female.

The mothers of the rats of experiment 2 were fed the selenium diet 5 to 7 days (group 1) or 15 to 19 days (group 2) before giving birth, respectively, or 5 days before mating and during the experimental period (group 3). Twenty animals were used in each experimental group.

For experiment 3, two groups of female stock rats were mated simultaneously; one was fed the stock ration and the other was given the seleniferous diet 5 days prior to mating and until birth of the litters. The litters from group 1 were given for nursing to females of group 2 within 24 hours after birth; in turn, the young born of the mothers of group 2 were nursed by group 1 mothers. Both groups received the stock ration after the birth of the litters and for the duration of the experiment. They totaled 22 young rats.

In experiment 4, three groups of rats were fed a diet containing 10 ppm selenium from sesame. Two groups had been bred and kept on the stock ration; the third group was fed the 4.5 ppm seleniferous diet. These animals were 3, 6 and 4 weeks old at the beginning of the experiment and consisted of 14, 18 and 16 animals, respectively.

Fifteen 4-week-old rats, bred on the 4.5-ppm selenium diet and 20 stock rats kept for 4 days on the 10-ppm selenium diet were used in experiment 5. It was expected from the results of the previous experiments that both groups would have similar liver selenium levels. The rate of elimination of liver selenium was compared while both groups were fed the stock ration.

RESULTS

The amounts of selenium detected in liver with the analytical technique involving drying may be smaller than that existing in the fresh organ, because, according to Heinrich and Kelsey (5), volatile selenium compounds may exist. Therefore, the present results refer to the nonvolatile liver selenium as the amount of volatile selenium was not determined.

The liver selenium levels in the two groups of rats of experiment 1 changed in the opposite direction with time (fig. 1). In one group the amount of selenium rose from 1.7 to 3.8 ppm; in the other it fell from 6.3 to 3.2 ppm. The graphic representation of the corresponding values falls on two straight lines which cross each other at the point corresponding to day 87 of the experiment. No gross differences in weight gain or food intake were observed between these groups. The individual selenium values of animals of one group killed at the same age fall generally within the range of ± 0.4 ppm.

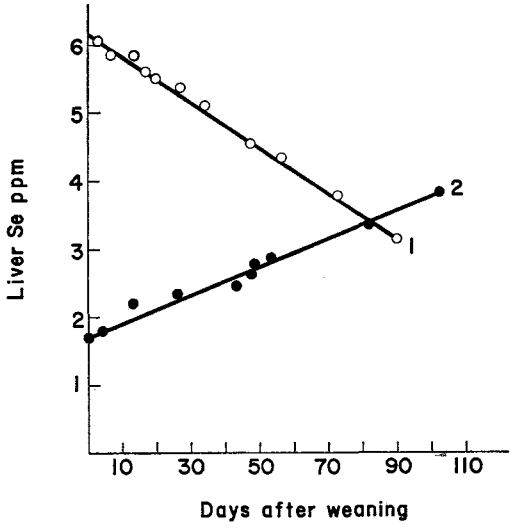

Fig. 1 Experiment 1. Liver selenium levels in rats fed a diet with 4.5 ppm selenium. Group 1: rats bred on the selenium containing diet; group 2: rats bred on the stock diet.

The influence of duration of feeding the seleniferous diet before the birth of the litters, on liver selenium at the time of birth and later, was studied in experiment 2; the results are summarized in figure 2. The young of group 1, born to dams fed the selenium-containing diet 5 to 7 days before giving birth, had at first the lowest liver selenium contents, which rose until they reached higher levels than those in the other series. The mothers of group 2 had been fed the experimental diet for about 2 weeks. The livers of their young had more selenium at birth, the amount of which rose more slowly than in the first series. The last group was comparable to group 1 of experiment 1 and consisted of animals born from mothers which had ingested the experimental ration 5 days prior to breeding. In this case, the liver selenium was highest at birth and decreased steadily.

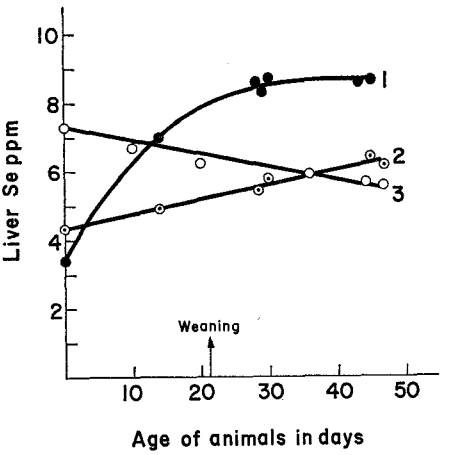

Fig. 2 Experiment 2. Liver selenium levels in young rats reared on a diet containing 4.5 ppm selenium. Group 1: dams put on the seleniferous diet 5 to 7 days before the birth of the litters; group 2: dams put on the diet 15 to 19 days before the birth of the litters; group 3: dams put on the diet 5 days prior to mating.

In experiment 3, litters were changed at birth between mothers fed the stock ration or the selenium diet, respectively. The liver selenium levels observed in figure 3 show a steep rise and subsequent fall in the litters of group 1 (nursed by dams fed selenium). In group 2 (rats born to mothers fed selenium and nursed by stock dams) these levels were high at birth and fell until leveling off at about 1 ppm.

Rats of experiment 4 were fed a diet containing 10 ppm selenium. The changes in liver values are shown in figure 4. In the stock animals these values increased to 14 ppm within 14 or 21 days, respectively, according to the age at which selenium feeding began, and fell later; in group 3, they rose steadily with no tendency to level off during the 52-day experimental period.

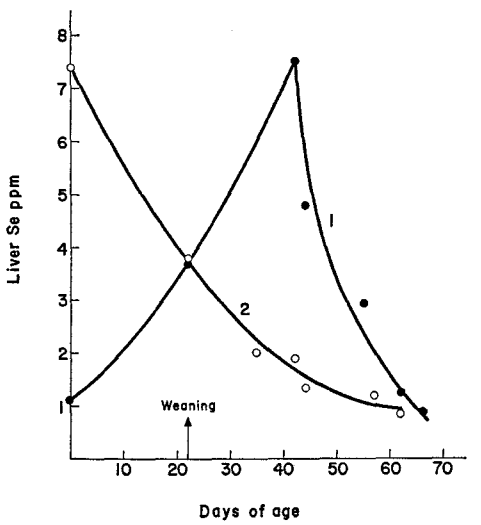

Fig. 3 Experiment 3. Influence of nursing on the liver selenium level of young rats. Group 1: bred on the stock diet, nursed by the mothers of group 2; group 2: bred on the seleniferous diet, nursed by the mothers of group 1.

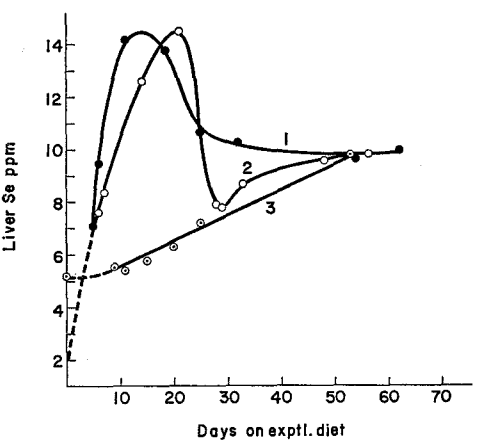

Fig. 4 Experiment 4. Liver selenium levels in rats fed a diet containing 10 ppm selenium. Group 1: stock animals 4 weeks old at the beginning of the experiment; group 2: stock animals 6 weeks old; group 3: animals bred on the 4.5 ppm selenium diet and 4 weeks old.

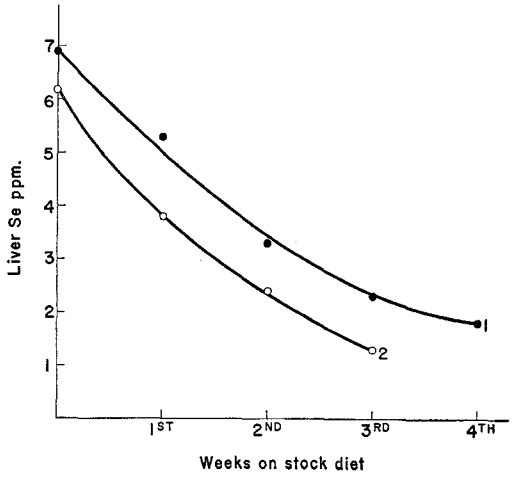

Fig. 5 Experiment 5. Liver selenium in rats fed the stock diet. Group 1: bred on the stock diet, fed for 4 days a diet with 10 ppm selenium; group 2: bred on the 4.5 ppm selenium diet.

The drop in liver selenium after the intake of this element had been discontinued was similar in rats born to mothers fed selenium and in stock animals previously fed a high selenium diet for 4 days (fig. 5, exp. 5). The level dropped from 6.2 to 1.3 ppm in 3 weeks in group 1 whereas it decreased from 6.9 to 2.3 ppm in the stock rats fed selenium.

DISCUSSION

The experimental diets were prepared with seleniferous sesame press cake, a material better suited for this type of investigation than inorganic selenium because it closely simulates the natural condition. The level of 4.5 ppm was chosen because it was the lower limit at which signs of intoxication became apparent in young stock rats under experimental conditions similar to those of the present experiment (1). The diets were supplemented with lysine because fewer symptoms of selenium toxicity developed in rats fed supplemented sesame rations than in those fed unsupplemented sesame rations (2).

The most important result of this study is that of experiment 1. It demonstrated that young rats may eliminate or accumulate selenium in the liver while consuming the same seleniferous diet, depending on the ration fed to their mothers (fig. 1). Those born from dams kept on the selenium-containing diet lost selenium from their livers, whereas rats bred on the stock ration accumulated this element under the same conditions. For the duration of the experiment these changes followed straight lines which crossed each other and showed no tendency for leveling off. This could mean that the animals in the two groups handled selenium metabolism in the livers in a different fashion, possibly because of differences in the intrauterine exposure to this element. Another possibility was that the handling of selenium may be related to the different levels existing at birth or the beginning of the experiment. The existence of an adaptation to selenium intake in rats born to dams fed the seleniferous diet during pregnancy was indicated by these results. It was not investigated whether the different protein levels in the stock diet and the seleniferous diet had any influence on the result.

It would be of interest to investigate how liver selenium will develop further in adapted animals on a seleniferous diet. In a preliminary trial with four second-generation rats, born to mothers which had been bred and kept on the 4.5 ppm selenium diet, the liver levels were much lower at birth than in the first generation, i.e., 4.7 compared with 7.1 ppm.

In experiment 2 the influence of intrauterine exposure on adaptation was studied by following the changes in the selenium contents in the livers of young rats from birth to about 6 weeks of age, and exposure to this element for different lengths of time during embryonic life. The course of change of the selenium levels with time depends on the selenium level at birth, which is, in turn, dependent on the time of feeding the seleniferous diet to the dams prior to the birth of the litters. The fast and discontinuous selenium accumulation in the rats of group 1 (fig. 2, exp. 2) with the lowest initial values was similar to that of the animals of groups 1 and 2 (fig. 4, exp. 4). In both cases the previous exposure to selenium had been less and the rise of the liver levels was faster than in the comparable groups of the respective experiments. Liver selenium increase was faster in rats of group 2 (fig. 2, exp. 2) whose mothers

received the experimental diet for 2 weeks during pregnancy, than in animals of group 1 (exp. 1) which had been bred on the stock ration and were fed the experimental diet after weaning. Perhaps this is related to the results of experiment 3 which gives evidence for a rapid increase of selnium in young rats during nursing. As the animals of this experiment were fed the stock diet, this rise was caused by the selenium excreted in the mother's milk. In experiment 2 this may have enhanced the selenium accumulation within the relatively short experimental period. Comparison with the performance of rats of group 1 (exp. 2) shows that some adaptation had taken place. Possibly liver selenium would level off if the duration of the experiment were extended. The selenium content in livers of the rats of group 3 (fig. 2) decreased at exactly the same rate as in experiment 1. The corresponding curves can be combined into one single, straight line. These results point to the influence of time of feeding the seleniferous diet to the mothers prior to the birth of their litters on the adaptation to selenium.

Experiment 3 was designed to investigate the course of the changes of liver selenium in young rats exposed to different intrauterine and dietetic levels of this element. A very rapid rise was evident in the animals nursed by dams previously fed the selenium diet and kept on the stock ration since birth of the litters, indicating high selenium excretion in the milk. Persistent rise of selenium level after weaning, when selenium intake stopped, points to liver deposition of selenium mobilized from other tissues.

After only 5 days of feeding a diet containing 10 ppm selenium to young stock rats, liver levels rose to over 6 ppm from an initial value of about 1 ppm. The maximum value of 14 ppm was reached earlier in younger animals than in older animals. The subsequent decrease may indicate that an adaptation to selenium intake may develop also under these conditions. The difference in performance between groups 1 and 2 (fig. 4) points to the importance of the age at which exposure to selenium occurred. The initial rise and subsequent fall of selenium in these animals brings to mind an observation of Rosenfeld and Beath (6) that tissue selenium is high in animals dead with blind staggers soon after the disease developed and considerably lower if the disease was prolonged. The rise in liver selenium in rats of this experiment, bred on the 4.5 ppm selenium diet, developed differently from that of the stock animals after the high selenium diet was fed. The corresponding values fell on a straight line in the graphic representation of figure 4 and indicate that these animals had adapted themselves to the selenium intake but could not handle the large intake as they handled that of experiment 1. A slightly lower selenium content (less than 10 ppm) would be more suitable for an experiment of longer duration because signs of intoxication became evident in some of the rats.

The outcome of the last experiment presented in figure 5 shows that rats adapted to selenium could eliminate this element at a slightly faster rate from the liver than the nonadapted controls. The difference, however, was too small to allow any conclusion on the physiological importance of this observation. Ganther et al. (7) observed that volatilization of a single dose of injected selenite was enhanced by prefeeding selenium. According to Hopkins and co-workers (8), urinary excretion also increased progressively with previous selenium intake, indicating an inverse relationship between selenium retention and body selenium under the conditions of a 2-week feeding period of the seleniferous diet. In these cases the experimental conditions were such that different levels of selenium should be expected to exist in the rats which were being compared. The animals of the experiment presented in figure 5 had similar amounts of body selenium as judged by the liver levels. The possibility that adapted rats absorb selenium at a lower rate than unadapted animals, or that differences in volatilization of selenium may exist between adapted and nonadapted animals, was not investigated.

Liver level may or may not reflect the total body selenium content under the present conditions. Hopkins et al. (8) found retention from a single dose of ^{75}Se similar in the livers of rats fed 5 ppm selenium for 2 weeks and in controls which had not been given selenium previously; carcass retention, however, was less in the former than in the latter. If these results, obtained un-

der very different conditions, can be applied to our experiments, it could be expected that carcass selenium between adapted and nonadapted rats differs more widely than liver selenium.

It cannot be concluded from the present experiments that a direct relationship exists between liver selenium and intoxication. The observation of Rosenfeld and Beath (6) on selenium in animals dead from blind staggers does not support the existence of such a relationship under the conditions of lethal intoxication in cattle. A connection between tissue selenium and intoxication may be expected, however, when the selenium intake is near the toxic level. Previous results from this laboratory (1, 2) pointed to the existence of an acquired tolerance to selenium through comparison of reproductive performance, growth, hemoglobin, hematocrit and body water in rats bred on a seleniferous or stock diet and fed a moderately toxic selenium-containing diet. Harr et al. (9) found median survival age greater in rats receiving diets with increasing selenium levels than in those receiving a constant level, but admit that their data are inconclusive concerning selenium tolerance. From observations of Tsuzuki et al. (10) it can be concluded that adaptation to selenium exists in species other than the rat. These authors exposed several generations of mice to selenium intake by inhalation. The animals bred under these conditions were less susceptible to toxic doses of this element than the controls.

The problems of the relationship between liver selenium and resistance to intoxication, the persistence of adaptation after the ingestion of selenium has been discontinued, the selenium balance in the adapted animals and the existence of similar mechanisms in other species, await further investigations and are of considerable interest in relation to chronic selenium ingestion in cattle and man.

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