THE EFFECT OF BROMOBENZENE ADMINISTRATION ON SELENIUM EXCRETION IN RATS [1]

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ABSTRACT

The economic impact of selenium (Se) toxicity is marked in some regions of South Dakota, and suitable means of control and protection are of interest. The objective of this study was to re-examine the effect of bromobenzene (BrB) on the excretion of selenium under various experimental conditions. Two groups of 5 rats each were fed wheat diets containing 5 ppm Se as sodium selenite and 2 groups were fed 5 ppm Se as seleniferous wheat. After an initial 2-week period, the Se-diets were replaced with a basal diet (0.24 ppm Se) and one each of the dietary treatment groups was administered a single oral dose of BrB (100 mg/kg) in corn oil. After eight days another single dose of BrB was administered to the same groups. The animals were then returned to the respective Se-diets and 6 consecutive daily doses of BrB (50 mg/kg) were administered to the same groups previously receiving BrB. Daily throughout the entire experimental period, 24-hr accumulated urinary Se excretion was measured. The results show that no significant changes in 24-hr Se excretion were observed, regardless of the level or form of Se or whether the BrB was administered as a single dose or repeated doses.

INTRODUCTION

Bromobenzene (BrB) was thought to be detoxified by conjugation with tissue cysteine and methionine and subsequently excreted in the urine (Stekol, 1937). Consideration of that knowledge led Moxon et al. (1940) to administer BrB to selenized animals in an attempt to protect them. These workers observed that steers on seleniferous range greatly increased their selenium excretion when given BrB orally. Rats fed selenium diets (Moxon and Olson, 1940; Dinkel et al., 1957) and dogs fed 10 ppm Se as seleniferous corn (Moxon et al., 1940) were reported to respond to BrB administration, but specific data were not given. Lemley (1940) and Lemley and Merryman (1941) have also reported large responses in Se excretion following the administration of BrB to humans, who were presumably consuming large amounts of Se.

On the other hand, Westfall and Smith (1941) studied the effect of BrB on Se excretion in rabbits and found no significant

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effect. More recently Tajita (1959) demonstrated that subcutaneously administered BrB caused a several-fold increase in selenium excretion by selenized rabbits. However, other work from the same laboratory (Sakurayama, 1960) showed no effect of the compound on Se excretion by rabbits.

Relative to the proposed mechanism for the BrB effect that Se is detoxified by excretion as the Se analog of mercapturic acid, McConnell et al. (1959) showed that administration of BrB increased the amount of Se-75 activity in the mercapturic acid fraction. However, the evidence was based solely on qualitative chromatographic data. No attempt was made to provide quantitative data, and so the relative importance of the pathway remains unknown.

Since the published data show considerable variation in response of selenized animals, it is the purpose of this study to evaluate once again the effect of bromobenzene on Se excretion under a variety of conditions using the rat as the experimental animal.

EXPERIMENTAL

Twenty male Sprague Dawley rats, averaging 82 g, were divided into 4 groups and placed in individual stainless steel hanging wire cages. The rats were fed a diet with the following composition (%): wheat, 79.5; casein, 12; yeast, 2; U.S.P. Salts XIV[2] 3; vitamin fortification mixture[2], 0.5; and corn oil, 3. For 2 groups, Na_2SeO_3 was added at an approximate level of 5.0 ppm (4.7 ppm total Se by analysis). For the other two groups, high-Se wheat was substituted for regular wheat to give approximately 5 ppm Se (5.7 ppm by analysis).

The rats were fed *ad libitum* for 2 weeks to reach a stabilized state relative to Se excretion. The animals were placed in stainless steel metabolism cages and 24-hour urine samples were collected on the final 3 days of the adjustment phase (days 12, 13 & 14).

On the 15th day, the seleniferous diets were replaced by basal diets of the same composition but containing no added Se (0.24 ppm Se). One group previously receiving Se-wheat, and one group previously receiving selenite were each administered a single dose of BrB (100 mg/kg body weight) in corn oil by stomach tube. An equal amount of corn oil was given to the two control groups. Urine collections were continued for 7 consecutive days (days 15 through 21). Urinary Se excretion had leveled off at a relatively low level (3-7 mcg/day) by the end of this period. In order to measure the effect of BrB on body stores of Se at low levels of intake, another single dose of the compound (100 mg/kg) was administered to the same groups on day 22 and urine collected for 3 consecutive days.

On day 25, all groups were returned to their respective selenium diets for the remainder of the experiment. After four days, the animals which had previously been dosed with BrB, were given 6 consecutive daily doses of the compound at half the level of previous doses (50 mg/kg).

Se content of the urine was determined by the fluorometric method of Olson et al. (1975). The results were analyzed statistically (Steel and Torrie, 1960) by fitting the data for each treatment to polynomial regression lines within 4 segments of time: days 15-21, days 15-24, days 22-24 and days 29-34.

RESULTS AND DISCUSSION

The effects of BrB on Se excretion by rats fed Se as selenite are shown in Fig. 1, and the data for rats fed seleniferous wheat are shown in Fig. 2. Se excretion in the initial adjustment phase of the experiment (12-14 days) was higher for rats fed Se-wheat than for those fed selenite. This was also true for the final 10 days, when the animals were again fed the high-Se diets. The difference can probably be attributed to the difference in the Se level of the diets (4.7 ppm Se in the selenite diet vs 5.7 ppm Se in the seleniferous wheat diets.

The removal of all animals from the high-Se diets on the 15th day resulted in a rapid drop in Se excretion. The administration of a single dose of 100 mg of BrB per kg body weight did not affect the rate of decline of Se excretion. The level of excretion plateaued about the 20th day at 3.5 mcg/24-hr for the animals fed selenite and about 7.2 mcg/24-hr for the animals fed Se-wheat. Administration of another single dose of BrB on day 22 caused no significant change in Se excretion in either the selenite or Se-wheat groups.

When the animals were returned to their respective high-Se diets (day 25), there was still no significant effect of BrB administration even though 6 successive doses (50 mg/kg) were given. Slight numerical effects can be seen but, in most instances, rats receiving BrB excreted less Se than the control animals. These small decreases were probably due to a decrease in feed (Se) intake. This is reflected in the body weights, which tended to be lower for the groups receiving BrB (Table 1), especially in the final week. Thus the administration of BrB in single doses to animals just removed from high Se intakes or maintained on low Se intakes, or the administration of multiple doses to animals on high Se intakes did not significantly increase the excretion of Se in the urine regardless of the form of Se being fed. Essentially the same results were observed in another similar trial (unpublished results).

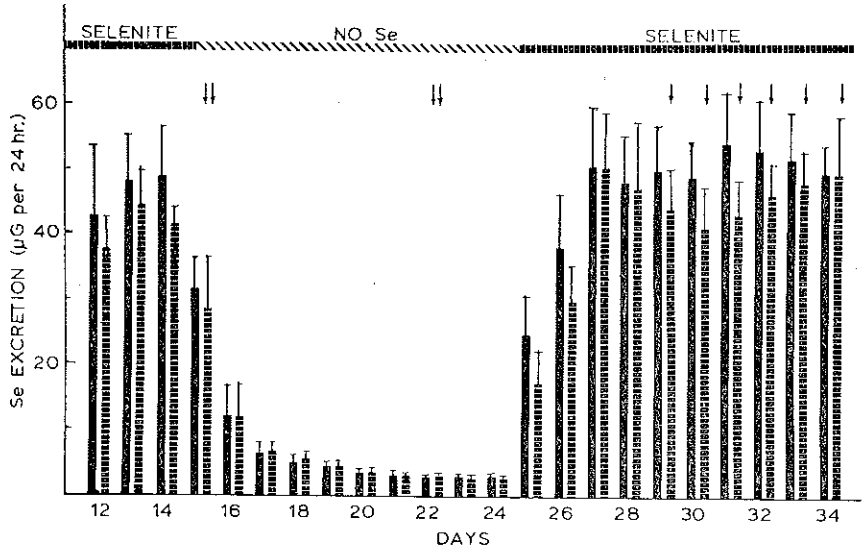

Figure 1. Effect of bromobenzene on the urinary excretion of Se by rats fed 5 ppm Se as selenite. The solid vertical bars represent Se excretion by the controls whereas the broken bars represent the Se excretion by rats which were given bromobenzene at specified times (arrows). Standard deviation is shown above each bar. Each arrow represents a dose of 50 mg of bromobenzene/kg body weight. There were 5 rats in each group. The horizontal bar at the top of the figure shows the dietary regime.

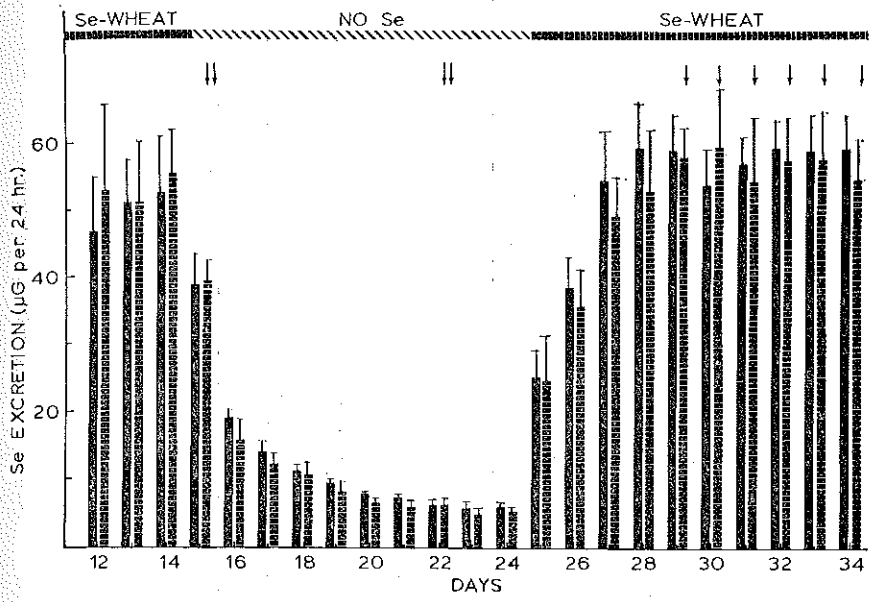

Figure 2. Effect of bromobenzene on the urinary excretion of Se by rats fed 5 ppm Se as seleniferous wheat. See fig. 1 for the explanation of the bars and arrows.

TABLE 1

Body Weight (g) of Rats on Various Diets With Occasional Bromobenzene Administration

Treatment	Days on Experiment					
	0	7	14	21	28	35
Se-Wheat Control	82 ± 6	132 ± 15	178 ± 21	226 ± 23	256 ± 27	282 ± 28
Se-Wheat + BrB	82 ± 6	134 ± 3	175 ± 12	213 ± 16	233 ± 20	250 ± 16
Selenite Control	82 ± 5	133 ± 9	175 ± 12	218 ± 22	247 ± 25	274 ± 28
Selenite + BrB	82 ± 3	127 ± 4	168 ± 6	210 ± 12	245 ± 19	254 ± 23

The variance of the results of our study from some of those reported by others earlier remains unexplained. The methods of analysis used in the 1940's were much less sensitive than the currently used fluorometric method, and it is conceivable they provided misleading results. A more likely explanation is that in the studies by Moxon et al. (1940), and those of Lemley (1940), urinary Se excretion was reported on the basis of concentration rather than total 24-hr excretion. In another study reporting beneficial effects of BrB (Lemley and Merryman, 1941), it was reported that patients were instructed to collect 24-hr samples, but no urine volumes were reported, and so it is not possible to judge whether or not total Se excretion was actually measured. Under such conditions, any fluctuation in total urine volume could result in rapidly changing Se concentrations. Since the experimental subjects in the early studies were subjected to repeated doses, it is conceivable they may have varied from their normal food and liquid intakes and thereby exhibited transitory changes in urinary Se concentrations. The early work by Westfall and Smith (1941), reported data on the basis of 24-hr excretion and describes results very similar to those reported in this study.

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