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Biological Activity of Trimethylselenonium-selenium and Influence of Vitamin E on its In vVivo Formation in Rats Using a Torula Yeast Diet

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BIOLOGICAL ACTIVITY OF TRIMETHYLSELENONIUM-SELENIUM AND INFLUENCE OF VITAMIN E ON ITS IN VIVO FORMATION IN RATS USING A TORULA YEAST DIET

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Chemistry Department Date

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INTRODUCTION[1]

During recent times, many efforts have been extended to investigate the metabolism of selenium compounds in microorganisms, animals and plants. Several investigators (1-3) have proposed some hypothetical metabolic pathways for selenium. However, the role of selenium in the life function of animals remains unknown.

Liver necrosis, as induced by the feeding of a Torula yeast diet which is unsupplemented with vitamin E and selenium, is known to be a fatal symptom in rats (4-6). The addition of either vitamin E or selenium to the diet is associated with the prevention of this condition. Schwarz (7) has suggested that vitamin E and selenium play their effect on the same syndrome by acting on alternate pathways of one stage in energy metabolism, thus enabling either substance to promote the over-all reaction. Tappel (8) has proposed that either substance acts to stabilize unsaturated lipids in the intact animal, i.e., as antioxidant in preventing lipid peroxidation and accompanying cellular damage. The report that the necrogenic syndrome may occur when rats are fed a diet low in polyunsaturated fat (9) does not appear to support the explanation offered by Tappel.

Little information is available about the effect of vitamin E on selenium metabolism in animals. In an experiment with rats, Hopkins[2]

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[1] The writing style of the Journal of Nutrition is adopted as a guide in preparing this thesis.

[2] Hopkins, L. 1962 Ph.D. Thesis, University of Wisconsin.

observed that vitamin E-deficient and vitamin E-supplemented animals which were given a small dose of selenium-75 both showed similar accumulation of selenium in the tissues and excretion in the urine. In a chick study, Jensen et al. (10) found that supplementing a Torula yeast basal diet containing 1 ppm of selenium with 20 IU of vitamin E per gram did not change the pattern of selenium retention in the tissues. While these results indicated a negative relationship between selenium metabolism and the presence of vitamin E in the diet, it is recognized that the measurements were not concerned with especial selenium metabolites.

At the present time, the important reactions of selenium in the animal are not understood. Various selenium substances have been detected in small amounts in biological systems, but these substances are analogues of important sulfur metabolites so it has not been possible to conclude that they have individual functions. These substances include selenomethionine (11-13), selenocysteine (13-15), selenocystine (11,16), selenotaurine (17), Se-methylselenocysteine (18), selenocoenzyme A (19), selenocystathionine (16,20), adenosine phosphoselenate (21,22) and Se-adenosylselenomethionine (23,24). However, some information about the end products of selenium metabolism is now evident. McConnell and Portman (25) have demonstrated that dimethylselenide occurs in the breath of rats injected with a high dose of selenate-Se. Palmer et al. (26) have observed that the trimethylselenonium ion[3]

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[3] Hereafter referred to as TMSe moiety or TMSeCl.

(TMSe) is a major metabolite of selenium in the urine of rats injected with varied levels of selenite or else fed a selenite-supplemented diet. Byard and Baumann[4] have made somewhat similar observations. Palmer[5] has further observed that other compounds of selenium, i.e., selenate and selenomethionine, are sources of the urinary TMSe ion and that other animals (turkeys) also excrete it. Thus, it is now possible to conclude that methylated selenium is a major end product of selenium metabolism. However, information about whether a form such as the TMSe ion has biological activity is not established. It may be significant that recent work by Obermeyer[6] indicates that TMSeCl is relatively non-toxic to the rat, i.e., a detoxified form of selenium.

In the experiments of the author, the biological activity of TMSeCl was tested and compared with the known activity of sodium selenite and of vitamin E with regard to the prevention of liver necrosis and death in the rat. The in vivo metabolic reactivity of TMSe ion was also studied by injecting Se^{75}-labeled TMSeCl into rats fed necrogenic (necrosis producing) and non-necrogenic diets and then observing the form(s) of selenium in the urine by means of paper chromatographic and autoradiographic techniques. The effect of the necrogenic syndrome on selenite-Se^{75} metabolism by the rat was also investigated by similar methods.

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[4] Byard, J. L. and C. A. Baumann 1968 Fed. Proc., 27:417 (abstr. 1146).

[5] Palmer, I. S. In preparation. South Dakota State University

[6] Obermeyer, B. D. 1969 M.S. Thesis, South Dakota State University.

EXPERIMENTAL

Biological Activity of Dietary Trimethylselenonium-Se

Male albino rats[7] weighing approximately 40-50 g at 3 weeks of age were used. All animals were fed a Torula yeast basal diet (table 1) for 1 week to partially deplete them of selenium and vitamin E reserves. They were then allotted at random into the control group (basal diet) and treatment groups which were fed the basal diet plus each of the following 6 different supplements: 0.15 and 1.50 ppm of chemosynthetic[8] TMSeCl-Se, 0.15 and 1.5 ppm of biosynthetic[8] TMSeCl-Se, 0.15 ppm sodium selenite-Se and 120 ppm vitamin E. The diet and water (deionized) were allowed ad libitum in this experiment as well as in other experiments.

The extent of survival and of liver necrosis of the animals fed the TMSeCl diets was compared with that of the animals fed either the selenite- or vitamin E-supplemented (protective) diet (7). Non-survival was marked by an acute illness at 3-4 weeks from the beginning of the feeding of the Torula yeast diet. The rats were generally sacrificed at the acute stage, the livers being removed and examined visually for necrotic areas (27). After one month from the

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[7] Albino rats used in this and other experiments were purchased from Sprague-Dawley, Inc., Madison, Wisconsin 53711.

[8] Both chemosynthetic and biosynthetic TMSeCl were prepared according to Palmer et al. (26).

TABLE 1

Composition of Torula yeast basal diet[1]

	%
Torula yeast	30.0
Sucrose	58.8
Lard, vitamin E-free	5.0
Salt mixture[2]	4.0
Vitamin mixture, vitamin E-free[3]	2.2

[1] All ingredients are supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.

[2] Salt mixture as described by Bernhart and Tomarelli (28).

[3] Vitamin diet fortification mixture in dextrose. The vitamin mixture supplied the following per 100 g of diet: vitamin A, 1980 IU; vitamin D, 220 IU; and (in mg) ascorbic acid, 99; inositol, 11; choline chloride, 165; menadione, 4.95; p-aminobenzoic acid, 11; niacin, 9.9; riboflavin, 2.2; pyridoxine·HCl, 2.2; thiamine·HCl, 2.2; Ca pantothenate, 6.6; and (in μg) biotin, 44; folic acid, 198; vitamin B_{12}, 2.97.

start of this experiment, the remaining rats were also sacrificed to facilitate examination of their livers.

Metabolic Reactivity of Injected Trimethylselenonium-Se

Male albino rats of 40-50 g initial weight were fed the basal diet (table 1) for 7 days. They were then allotted at random into two groups, one being continued on the basal diet and the other being fed the basal diet plus 120 ppm of vitamin E. At the time when lethal symptoms became evident with the animals fed the basal diet (see previous experimental section), the animals of both treatments were each injected intraperitoneally with Se^{75}-labeled[9] TMSeCl supplying 12 μg of selenium and 4.2 μC of radioactivity per 100 g of body weight of an animal. This dose was in 0.25 ml of solution. The animals of both treatments were fasted 12 hours before the injection and 6 hours afterward. Immediately on injection the animals were placed individually in stainless steel metabolism cages which allowed collection of

[9] TMSeCl-Se^{75} was prepared according to Palmer et al. (26) with a slight modification. Urine was collected from rats injected with a high dose of sodium selenite-Se^{75}. Radioactive TMSe-reineckate was obtained directly from the urine (29). This TMSe-reineckate salt was then converted to TMSeCl-Se^{75} by using a AG2-X8 resin (200-400 mesh, chloride form). Finally TMSeCl-Se^{75} was repeatedly purified by using paper chromatography along with autoradiography. This pure TMSeCl-Se^{75} was mixed with carrier chemosynthetic TMSeCl to give the injection solution, each ml containing 48 μg of selenium (sp. act. 0.35 μC/μg).

the urine. The mean body weights of the animals fed the basal diet and the basal diet plus vitamin E were 101 and 109 g, respectively, at this time. The urine was collected at room temperature in 12 ml centrifuge tubes. The collection periods covered intervals of 0-12, 13-24 and 25-144 hours. The urine samples were centrifuged in a Sorvall RC-2 automatic refrigerated centrifuge. The volume of supernatant was measured. The supernatant was stored in a freezer prior to measurement of the total Se^{75} activity and the separate activity of entities on the chromatogram.

The total Se^{75} activity was measured on all samples of urine. A 50 µl portion of each was counted with a Packard well-type auto-gamma spectrometer. A given portion of the injection dose of TMSeCl-Se^{75} was similarly counted and then the per cent of the dose represented in each sample was calculated.

The separate activity of chromatographic entities was studied as follows: 50 µl portions of each first 12-hour sample were applied to duplicate sheets of Whatman No. 1 chromatographic paper (46 × 57 cm). Each paper was then developed in the first dimensional solvent (phenol:water, 73:27, w/w) for approximately 16 hours with a traveling distance of 40 cm. After being dried in a 70°C oven, the chromatogram was further developed in the second dimensional solvent (butan-1-ol: acetic acid:water, 4:1:1, by vol.) for 10 hours with an approximate 30 cm traveling distance. The chromatogram was then placed underneath a Kodak no-screen medical X-ray film (35.6 × 43.2 cm) for sufficient time to allow appropriate exposure. Any discrete major spots from Se^{75}

activity were located on the film following development. These areas were outlined exactly on the chromatogram to facilitate their removal and the measurement of their Se^{75} activities by the gamma counting methods. The position of the primary spot, designated as U-1, was at the same time used as the reference ($R_{U-1} = 1$) in calculating the R_{U-1} values of other major spots, if present. To facilitate the counting of other areas of the paper in a reproducible manner, the paper was sectioned at lines corresponding to R_{U-1} values of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.1 along the first solvent movement and at values of 0.5, 1.0, 1.5, 2.0 and 2.4 along the second movement. The divided parts of the chromatogram were also counted for Se^{75} activity. Their activities along with those of the separate major spots were calculated in terms of percentage of the activity in the urine samples.

Influence of Vitamin E on Trimethylselenonium Formation

The procedures used in this experiment were similar to those of the preceding section except as described below. A commercial $H_2Se^{75}O_3$ solution[10] was adjusted to pH 6 with sodium hydroxide and then carrier Na_2SeO_3[11] was added in order to give 10 μg of selenium and 80 μC of radioactivity per 0.2 ml of final solution, this volume being

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[10] This was a highly radioactive selenium-75 as $H_2Se^{75}O_3$ in 0.5 N HCl (508 mC/mg Se). It was purchased from the Nuclear Science Division, International Chemical and Nuclear Corp., Pittsburgh, Pa. 15236.

[11] Sodium selenite was prepared as described by Trelease and Beath (30) and was pure as determined by Olson et al. (31).

injected per 100 g of animal weight. Thus, labeled sodium selenite-Se^{75} was injected instead of labeled TMSeCl-Se^{75} in this instance.

RESULTS

Biological Activity of Dietary Trimethylselenonium-Se

The data regarding the effect of dietary TMSeCl-Se supplements on the necrogenic syndrome of rats are shown in table 2. In two trials where dietary selenite-Se (0.15 ppm) or vitamin E (120 ppm) protected completely against mortality and liver necrosis during a 30-day experimental period, chemosynthetic TMSeCl-Se at levels of 0.15 and 1.50 ppm gave no definite protection. Likewise, these levels of biosynthetic TMSeCl-Se gave no protection in one trial. The similarity of the time-death relationship between animals fed the basal diet and the basal diet plus supplemented TMSeCl (fig. 1) was further evidence that TMSe was inactive against the necrogenic syndrome. It was of interest that whenever an animal survived the 30-day period when given the basal diet or the basal diet plus TMSeCl supplement, the liver showed mild necrosis although the animal had always appeared healthy during the experiment.

The appearance of typical non-necrogenic and necrogenic livers is shown in figure 2. A liver showing no necrosis, as was the case with animals fed sodium selenite-Se or vitamin E, is illustrated in photograph A. Livers showing necrosis, as was the case with animals fed the basal diet and the basal diet plus TMSeCl-Se, are illustrated in photographs B and C, respectively.

TABLE 2

Effect of TMSeCl-Se in preventing mortality and liver necrosis with rats fed a Torula yeast diet

Supplement	Level	Exp. no.	% Mortality[1]	Liver necrosis
	ppm			
None		1	86	+[2]
		2	100	+
Chemosyn. TMSeCl-Se	0.15	1	86	+[2]
		2	100	+
Chemosyn. TMSeCl-Se	1.50	1	86	+[2]
		2	86	+[2]
Biosyn. TMSeCl-Se	0.15	2	86	+[2]
Biosyn. TMSeCl-Se	1.50	2	100	+
Sodium selenite-Se	0.15	1	0	-
		2	0	-
Vitamin E	120	1	0	-
		2	0	-

[1] Seven rats used for each group of both experiments with 30-day period.

[2] The last surviving rat was killed on terminal date. Its liver also showed slight necrosis. Sign (+) indicates necrotic syndrome while sign (-) indicates negative result.

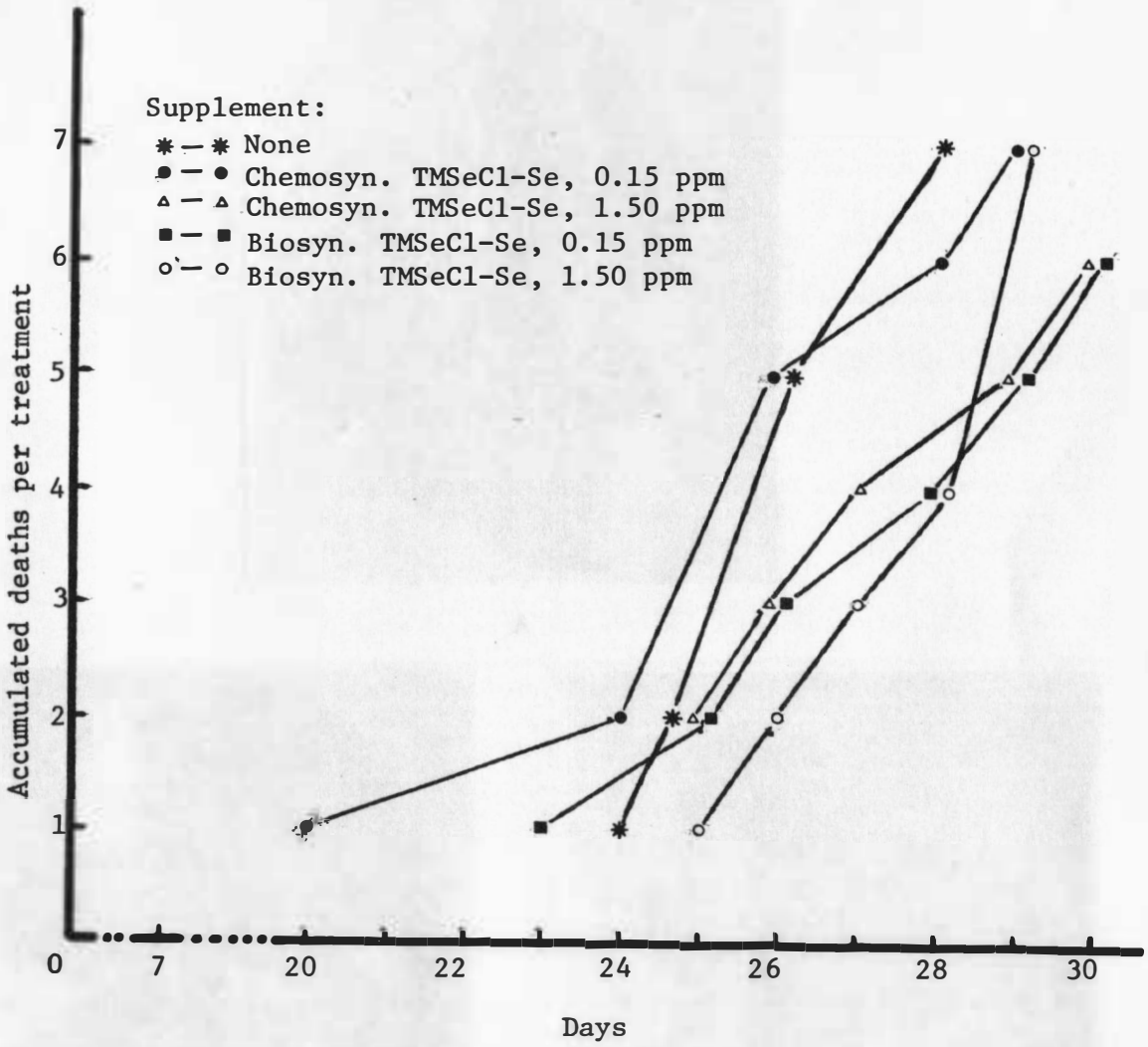
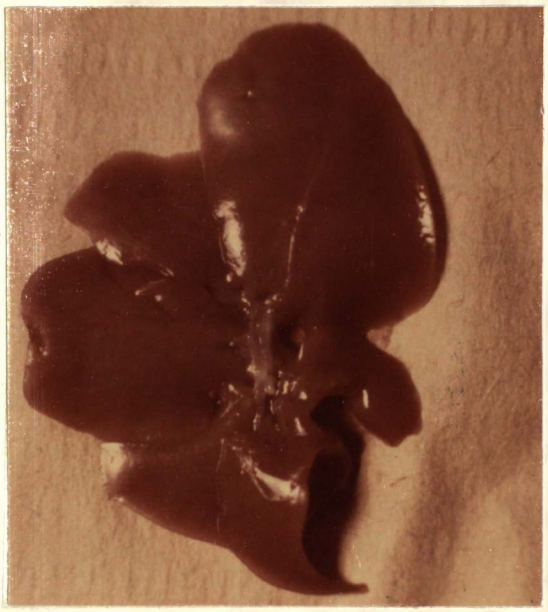
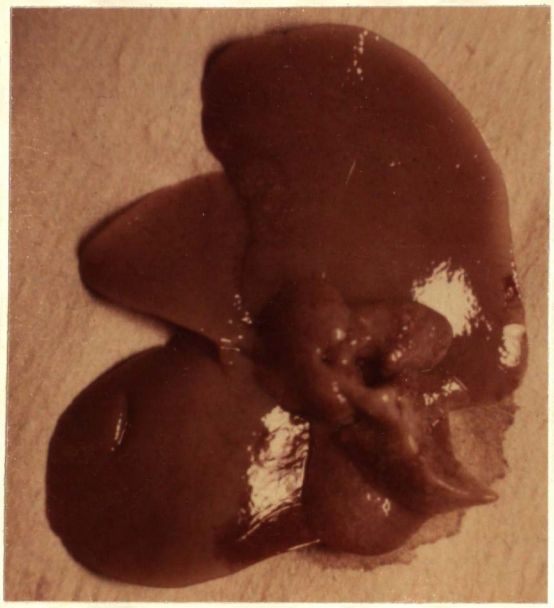


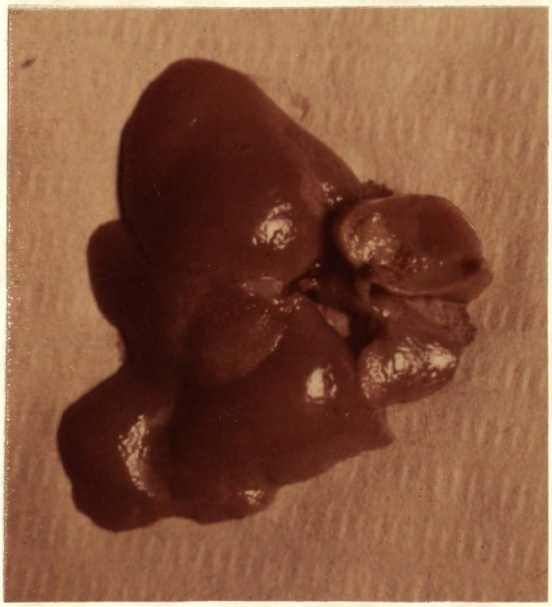
Fig. 1. Time-death relationship with rats fed the Torula yeast basal diet and the basal diet plus TMSeCl supplements. All rats were fed the basal diet for 7 days. They were then grouped and fed the respective treatment diets.



A



B



C

Fig. 2. Normal liver (A) in contrast to necrotic livers (B,C). A. Basal diet plus 120 ppm vitamin E or 0.15 ppm Se as sodium selenite. B. Torula yeast basal diet. C. Basal diet plus 0.15 or 1.50 ppm Se as trimethylselenonium chloride.

Metabolic Reactivity of Injected Trimethylselenonium-Se

The extent to which Se^{75} activity from injected TMSeCl-Se^{75} appeared in the urine of rats fed either the basal diet (necrogenic) or the basal diet plus vitamin E (non-necrogenic) is shown in table 3. About 70% of the activity of the dose was excreted in the urine during the first 12-hour period irrespective of which diet was fed. A smaller activity was excreted later so that the total amount excreted over 144 hours amounted to about 82%. It was noteworthy that some animals excreted almost all of the dose over the 144-hour period with two animals excreting 95% of the total dose in that time. Thus, the injected TMSe-Se^{75} was poorly retained when administered at rather low levels to animals, some of which were known to be deficient in factors protecting against the necrogenic syndrome, i.e., selenium or vitamin E.

The finding that the injected TMSe-Se^{75} appeared in only one substance in the first 12-hour urine is shown on a typical autoradiogram with a rat fed the basal diet (fig. 3). Similar results were obtained with animals fed the basal diet plus vitamin E. The single spot was designated as U-1 (26). On counting the Se^{75} activity of this area on the chromatogram, it was determined that the single spot accounted for all of the activity of the urine (table 4).

Along with the observance that urine of TMSe^{75}Cl-injected animals gave a single spot of radioactivity on the chromatogram in the area of TMSe (26), a further chromatography was used to characterize the identity of the spot. In the procedure, a 50 µl portion of urine containing the radioactive U-1 metabolite was mixed with 10 µl of the

TABLE 3

Urinary excretion of radioactivity of injected TMSeCl-Se^{75} with rats fed a Torula yeast diet unsupplemented and supplemented with vitamin E

Collection interval	Supplement	% of TMSeCl-Se^{75} dose[1] excreted		
		Exp. 1[2]	Exp. 2[3]	Avg.[4]
hours				
0----12	None	77.13 ± 2.81	64.30 ± 5.98	70.13 ± 4.54
	Vitamin E	73.20 ± 9.73	68.33 ± 1.53	70.54 ± 5.26
13----24	None	5.29 ± 0.76	3.95 ± 0.92	4.56 ± 0.85
	Vitamin E	5.04 ± 0.50	4.44 ± 0.64	4.71 ± 0.58
25---144	None	4.87 ± 1.38	9.81 ± 3.46	7.06 ± 2.31
	Vitamin E	8.12 ± 3.61	5.28 ± 0.63	6.57 ± 1.99
Total, 0-144	None	87.09 ± 3.01	74.49 ± 4.56	81.49 ± 3.70
	Vitamin E	86.36 ± 5.92	78.04 ± 1.47	81.83 ± 3.49

[1] Injection dose for rat per 100 g body weight: Exp. 1, 4.2 μC Se^{75} as H_2SeO_3 and 12 μg Se as Na_2SeO_3; Exp. 2, 10 μC and 10 μg Se, respectively.

[2] Mean ± SE of 5 rats.

[3] Mean ± SE of 6 rats.

[4] Weighed Mean ± SE of Exp. 1 and 2.

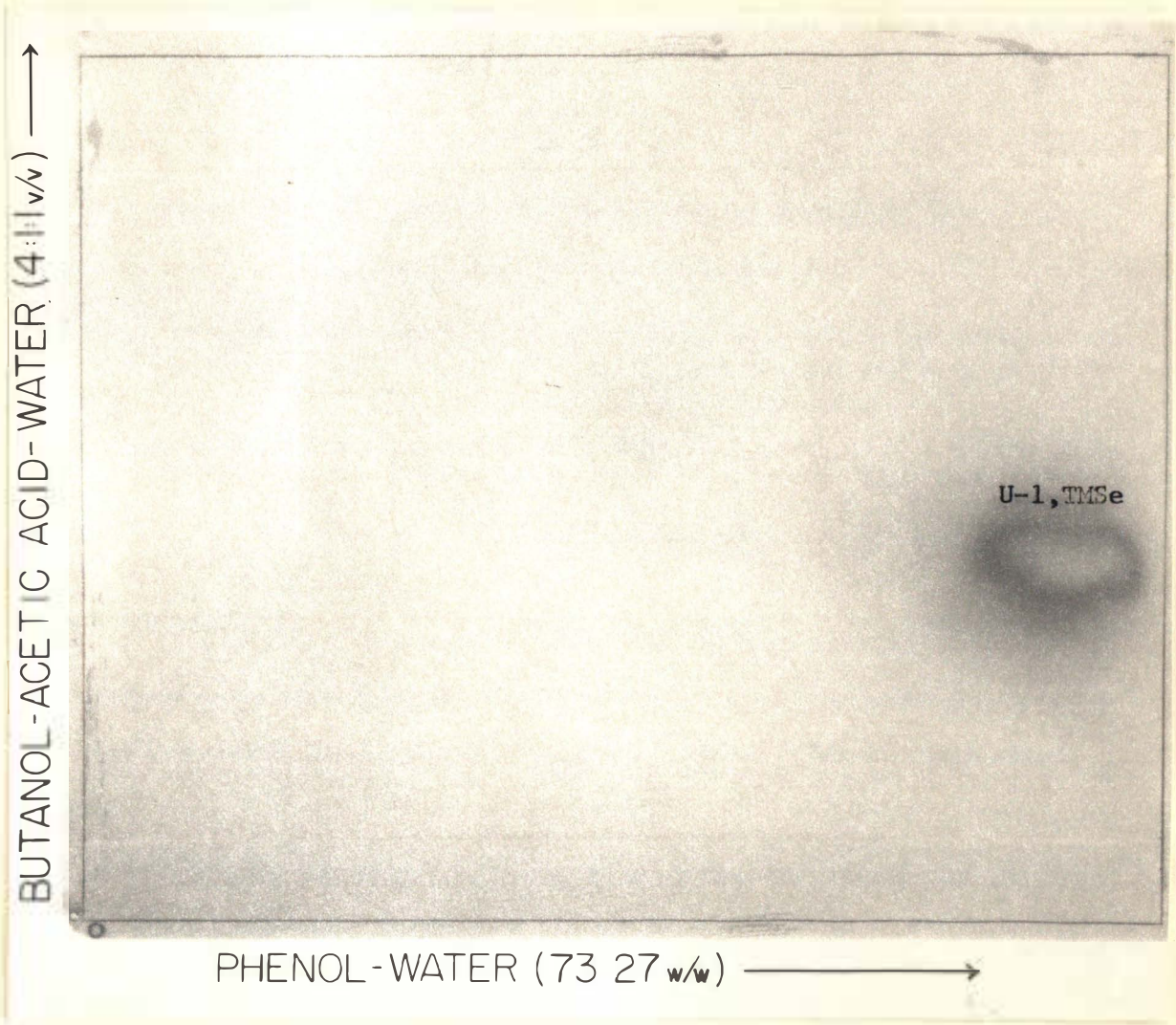


Fig. 3. Typical autoradiogram of two-dimensional chromatogram from urine sample of rats fed a Torula yeast basal diet or the basal diet plus vitamin E. Rats were injected with Se^{75}-labeled TMSeCl. Fifty microliters of the first 12-hour urine were applied to the origin. The designated U-1 was the only radioactive selenium metabolite in the urine and it was confirmed as TMSe. The phenol solvent was used first.

TABLE 4

Occurrence of urinary radioactivity as U-1 with rats injected with Se^{75}-labeled TMSeCl[1]

Diet	% activity of Se^{75} as U-1[2]	
	Exp. 1[3]	Exp. 2[4]
Basal	100.16 ± 1.58	100.61 ± 0.82
Basal + vitamin E	99.24 ± 1.14	99.26 ± 0.51

[1] Same animals as described in table 3 data; 0 to 12 hour urine samples.

[2] U-1 has a R_f value of 0.90 and 0.42 along the first and second solvent movement, respectively.

[3] Mean ± SE of 5 rats.

[4] Mean ± SE of 6 rats.

$TMSe^{75}Cl$ solution (biosynthetic) that was used for injection and with 10 μg of non-labeled TMSeCl (chemosynthetic). This preparation was then subjected to the chromatographic and autoradiographic procedures already described. Again, only one discrete spot appeared on the autoradiogram. The spot had a R_f value in agreement with that reported for TMSe (26). Thus, the activity of the designated U-1 and of the $TMSe^{75}$ were superimposed. When the chromatogram was sprayed with the Munier and Macheboeuf modification of Dragendorf's Reagent (32) as described by Palmer et al. (26), a single colored spot (pinkish orange) which was in the identical position of the spot on the autoradiogram was evident. Thus, the movement of the designated U-1, of biosynthetic $TMSe^{75}$ and of chemosynthetic TMSe on the chromatogram were identical, indicating that the injected TMSe occurred unchanged in the urine.

Influence of Vitamin E on Trimethylselenonium Formation

When selenite-Se^{75} was injected into rats fed either the basal diet or the basal diet plus vitamin E (non-necrogenic), the extent of Se^{75} activity excreted in the urine was somewhat low (table 5) in comparison with the higher and more rapid excretion which was observed when TMSeCl-Se^{75} was injected (table 3). In the case of the data shown in table 5, the dietary treatment was associated with an effect on the Se^{75} activity excreted in the first 12-hour period after the injection. This was particularly evident with the animals of experiment 1 where those fed the basal diet excreted 13% of the injected activity in the interval while those fed the basal diet plus vitamin E excreted 8%.

TABLE 5

Urinary excretion of radioactivity of injected Na_2SeO_3-Se^{75} with rats fed a Torula yeast diet unsupplemented and supplemented with vitamin E

Collection interval	Supplement	% of Na_2SeO_3-Se^{75} dose[1] excreted		
		Exp. 1[2]	Exp. 2[3]	Avg.[4]
hours				
0----12	None	13.04 ± 1.30	9.09 ± 1.65	10.88 ± 1.49
	Vitamin E	8.11 ± 0.78*	7.13 ± 1.48	7.57 ± 1.16
13----24	None	9.96 ± 0.66	3.66 ± 0.67	6.52 ± 0.66
	Vitamin E	7.96 ± 0.73	4.02 ± 1.01	5.81 ± 0.88
25---144	None	11.12 ± 0.91[a]	5.75 ± 1.27	7.90 ± 1.13
	Vitamin E	12.95 ± 2.02	5.49 ± 0.90	8.88 ± 1.41
Total, 0-144	None	34.93 ± 1.84[a]	18.50 ± 2.81	25.07 ± 2.42
	Vitamin E	29.02 ± 2.77	16.64 ± 2.87	22.27 ± 2.82

[1] Injection dose for both experiments: 80 μC Se^{75} as H_2SeO_3 and 10 μg Se as Na_2SeO_3 per 100 g rat.

[2] Mean ± SE of 5 rats, except the value with superscript "a" which is Mean ± SE of 4 rats.

[3] Mean ± SE of 6 rats.

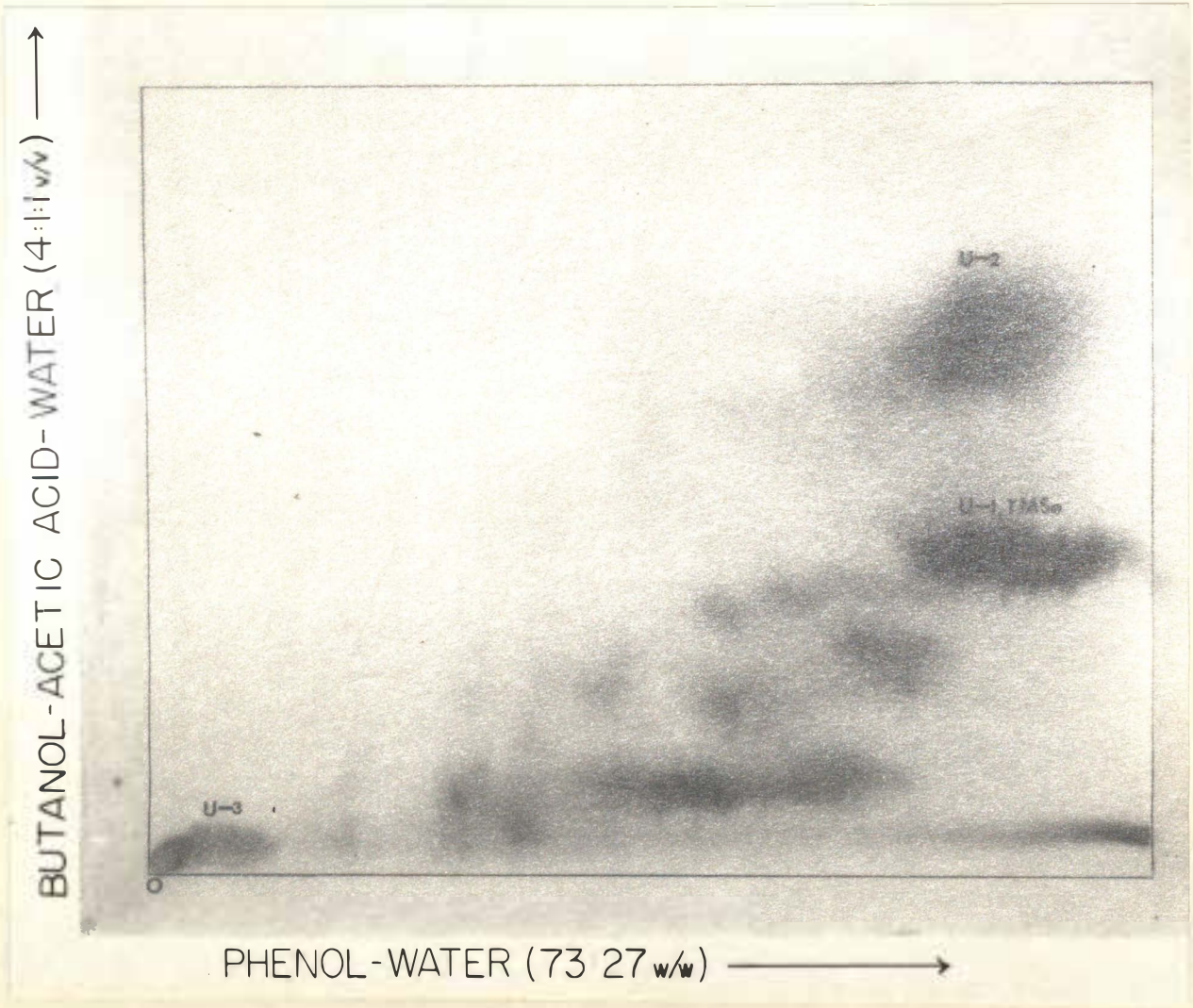
[4] Weighed Mean ± SE of Exp. 1 and 2.

* Indicates significant difference ($P < 0.05$) by t test (33).

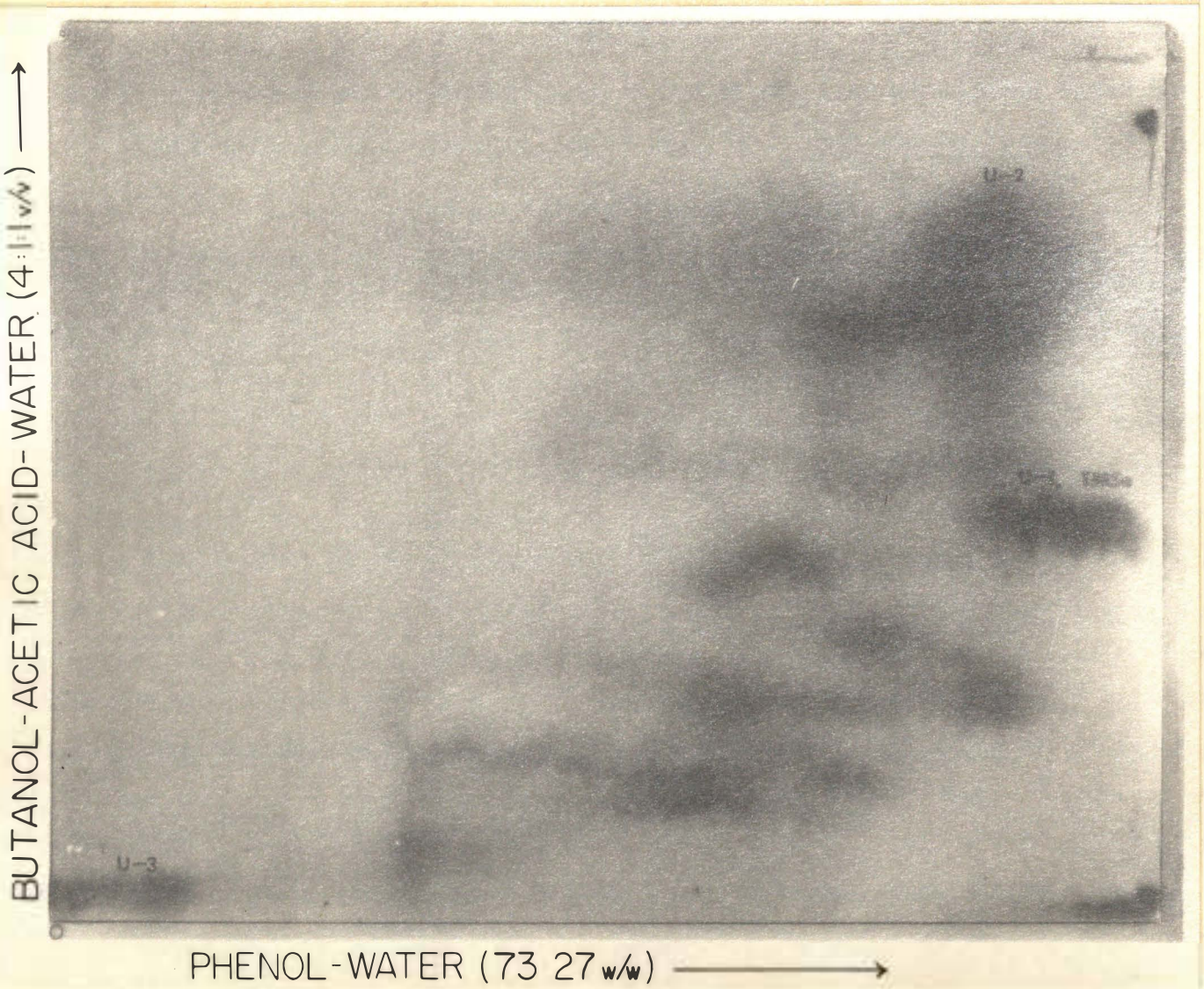
The Se^{75} measurements on later urine collections, i.e., over 13-24 and 25-144 hours, did not show significant treatment effects.

In contrast to the observation that the urine of $TMSe^{75}$-injected rats contained only the injected form of selenium, the first 12-hour samples of urine from $Na_2Se^{75}O_3$-injected animals were found to contain numerous forms of Se^{75} as observed with the chromatographic and autoradiographic methods (fig. 4). The most prominent spots on the autoradiograms were designated as U-1, U-2 and U-3, the first spot (U-1) corresponding in R_f value to TMSe. The respective R_f values for U-1, U-2 and U-3 were 0.90, 0.86 and 0.09 along the first solvent movement and were 0.42, 0.70 and 0.04 along the second solvent movement. These spots were present whether the animals were fed the basal diet or the basal diet plus vitamin E. The presence of vitamin E in the diet had an effect on the proportion of urinary Se^{75} activity represented by U-1 and U-2 but apparently not on the proportion represented by U-3. The results shown in table 6 at the bottom of the chromatogram chart give the values found in one experiment where the urinary Se^{75} as U-1 was reduced from 28 to 18% and that as U-2 was increased from 14 to 22% when the animals received vitamin E in the diet. However, the change in either substance did not result in a change of the total combined percentage of urinary Se^{75} from both substances, i.e., remaining about 40-42%. In terms of the total dose excreted in the first 12 hours, rats fed the basal diet excreted 2.53 and 1.26% as U-1 and U-2, respectively, while rats fed the basal diet plus vitamin E excreted 1.26 and 1.54%, correspondingly. Thus, the presence of vitamin E in

Fig. 4. Typical autoradiograms of two-dimensional chromatogram from urine sample of rats fed a Torula yeast basal diet (Photograph A, page 22) and the basal diet plus vitamin E (Photograph B, page 23). Rats were injected with Se^{75}-labeled Na_2SeO_3. Fifty microliters of the first 12-hour urine were applied to the origin. The designated U-1 (confirmed as TMSe), U-2 and U-3 were major urinary Se^{75} metabolites. (Note the similar distribution of minor areas of Se^{75} activity with the two chromatograms). The phenol solvent was used first.



A. From urine sample of rats fed a Torula yeast basal diet.



B. From urine sample of rats fed a Torula yeast basal diet plus vitamin E.

TABLE 6

Percentage of Se^{75} activity on chromatogram of the first 12 hr urine after injecting sodium selenite-Se^{75} with rats fed a basal diet (top figures) and a basal diet plus vitamin E (bottom figures)[1,2]

Relative $R_{U-1}{}^3$	0	0.2	0.4	0.6	0.8	1.0	1.1
2.4	0.08 ± 0.02 0.06 ± 0.02	0.05 ± 0.01 0.05 ± 0.02	0.06 ± 0.02 0.06 ± 0.03	0.11 ± 0.03 0.06 ± 0.03	0.15 ± 0.06 0.07 ± 0.03	0.09 ± 0.02 0.02 ± 0.01	
2.0	0.23 ± 0.04 0.36 ± 0.03	0.24 ± 0.05 0.47 ± 0.09	0.07 ± 0.14 1.49 ± 0.33	2.04 ± 0.42 4.49 ± 1.62	1.75 ± 0.47 1.64 ± 0.65	0.40 ± 0.06 0.50 ± 0.08	
1.5	0.09 ± 0.03 0.09 ± 0.02	0.25 ± 0.07 0.25 ± 0.05	1.03 ± 0.17 1.29 ± 0.30	3.19 ± 0.33 4.51 ± 0.75	3.61 ± 0.27** 5.13 ± 0.17	0.92 ± 0.14 0.65 ± 0.10	
1.0	0.10 ± 0.04 0.07 ± 0.01	0.65 ± 0.10 0.52 ± 0.10	2.23 ± 0.25 2.21 ± 0.37	5.53 ± 0.54 6.60 ± 0.57	4.90 ± 0.34 5.70 ± 0.37	0.40 ± 0.03 0.21 ± 0.02	
0.5	0.29 ± 0.14 0.08 ± 0.03	3.11 ± 0.25 2.55 ± 0.26	5.38 ± 0.22 4.74 ± 0.36	6.41 ± 0.40 6.32 ± 0.36	3.54 ± 0.07 3.22 ± 0.35	2.79 ± 0.40 1.65 ± 0.24	

Relative $R_{U-1}{}^3$

TABLE 6 (continued)

Percentage of Se^{75} activity in major spots excluded from above chart:

	U-1 spot	U-2 spot	U-3 spot
%	27.86 ± 1.49	13.86 ± 1.02	7.98 ± 1.73
	17.68 ± 3.37**	21.67 ± 1.75**	5.65 ± 1.54
R_{U-1}[4]	1.00	0.96 ± 0.01	0.10 ± 0.01
	1.00	1.67 ± 0.01	0.10 ± 0.00

[1] Same animals as described in Exp. 2, table 5; 0 to 12 hour urine samples.

[2] All figures are Mean ± SE of 6 rats.

[3] Relative R_{U-1} values are based on the central location of U-1 spot.

[4] The top and bottom figures represent relative R_{U-1} values in the direction of the first and second solvent movements, respectively.

**Highly significant difference ($P < 0.01$) by t test (33).

the diet was associated with an over-all decrease in the U-1 excretion and an over-all increase in the U-2 excretion. The other activity of the chromatogram, exclusive of that of U-1, U-2 and U-3, is shown for the designated areas of the chart (table 6). Except for a significant difference in one area representing only 4 or 5% of the urinary Se^{75} activity, the dietary treatment did not appear to affect the presence of the other selenite-derived selenium compounds of the urine. It was of interest to observe that the Se^{75} activity measured throughout the chromatogram accounted for essentially all of the activity which was present in the urine, so loss of any of the selenium by volatilization did not appear to be a factor.

DISCUSSION

The recent studies by Byard and Baumann[12], Palmer et al. (26) and Obermeyer[13] together with the results of this report enable new conclusions about selenite-Se metabolism in the rat. The identification of TMSe as a primary form of urinary selenium by Byard and Baumann[12] and Palmer et al. (26) established the concept that oxidized selenium is reduced in metabolism under ordinary conditions since the selenium was administered at both low and high levels. Previously, it was known that such selenium was reduced when administered at high levels since the breath contained volatile selenium as dimethylselenide (25). The determination by Obermeyer[13] that TMSe is relatively non-toxic when fed or injected while selenite is known to be highly toxic, was suggestive that the reduction-methylation process represents a detoxification reaction. Indeed, previous work with dimethylselenide has given results indicating that this form is also quite non-toxic (34). The reported findings of the author indicate that TMSe-Se differs from selenite-Se in that it does not react to form other urinary selenium compounds and is not biologically active. The previous report that an analogous form of sulfur, i.e., trimethylsulfonium chloride, is not a methyl source in animals (35) is of interest. Possibly dimethylselenide is also quite inactive in the animal since it has been reported that dimethylsulfide does not appear to be oxidized (36).

[12,13] See footnotes 4 and 6, respectively.

The identification of other forms of urinary selenium in rats injected with selenite would appear to be important. This is concluded from the observance in the present study (table 6) that animals affected with the necrogenic syndrome (without dietary vitamin E or selenium) appeared to show an abnormality in selenium metabolism. The effect consisted in a change in the ratio of the two most prominent urinary selenium substances, i.e., U-1 (TMSe) and U-2 (unidentified). Thus, U-1 and U-2 represented 28 and 14% of the urinary selenium with animals on the necrogenic diet and 18 and 22% of the urinary selenium with animals on the non-necrogenic (vitamin E supplemented) diet, respectively. It was noticeable that the animals on the necrogenic diet also excreted a greater total selenium (from selenite-Se^{75}) in the urine than did the animals on the non-necrogenic diet, especially during the first collection period after the administration of the selenium dose (table 5). From these results, it appeared that the rats showed an enhanced loss of selenite-derived selenium as TMSe when they were deficient in vitamin E under conditions when the necrogenic syndrome was imminent. The implications of these observations in the light of the knowledge that vitamin E and selenite-Se are each capable of preventing the necrogenic syndrome is exciting. Undoubtedly, knowledge about the structure of U-2 would be of great help in the further elucidation of the important reactions of selenite-Se metabolism.

SUMMARY

1. A Torula yeast diet was used to study the trimethylselenonium ion metabolism in the rat.

2. Dietary trimethylselenonium (as chloride salt) at 0.15 and 1.50 ppm levels of selenium was inactive in preventing liver degeneration in rats under conditions when 0.15 ppm of sodium selenite-Se or 120 ppm of vitamin E was active.

3. About 70% of the Se^{75} activity was recovered from urine in the first 12 hours after injecting rats with Se^{75}-labeled trimethylselenonium chloride. An 82% activity was obtained in a total 6-day period. Only one urinary Se^{75} metabolite was found and it was confirmed as trimethylselenonium ion. Vitamin E had no effect on these results.

4. The influence of dietary vitamin E on trimethylselenonium ion formation in vivo was investigated by injecting rats with sodium selenite-Se^{75} at a low level. While vitamin E-deficient animals showed a higher urinary excretion of selenium during the first 12 hours after injection, total excretion in 6 days was the same as those animals with vitamin E supplementation. When 120 ppm vitamin E was included in the basal diet, the urinary Se^{75} as trimethylselenonium metabolite was significantly decreased while that of another metabolite, U-2, was increased. The sum of these two major metabolites remained at about 41% of the first 12-hour urinary excretion of Se^{75} irrespective of the treatments.

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