1958

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TECHNICAL BULLETIN 20 MAY 1958

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Contents

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Introduction

The problem of selenium poisoning has been adequately described by several authors (13, 14, 21). Although the problem has been known for many years, the mechanism by which this element exerts its toxicity has not been clarified. As a result, what control measures are now available are of an empirical nature, and they fail to give the most desirable degree of protection.

In the search for an answer to the question of the mechanism of toxicity as well as for better control measures, the role of compounds containing biologically active methyl groups has been studied. As the review of literature which follows will reveal, some experimental work indicated that these types of compounds might indeed be involved in the metabolism of selenium. However, not all workers' data were in agreement here, and in view of such discord it was felt that further studies were needed.

The work reported here was carried on as part of an effort to clarify the role of the biologically active methyl group in the metabolism of selenium. The data presented deal with the protective effect of methionine and other biologically related compounds against selenium.

Review of Literature

Many workers have reported that a seleniferous diet of high protein content is less toxic than one of low protein content (7, 11, 13, 17, 19, 20). The chemical similarity of selenium and sulfur led to investigations to determine whether or not the sulfur-containing amino acids of proteins were responsible for the apparent protective effect.

Smith (19) reported that the results of the addition of 0.8% of DL-methionine to a diet containing 10 parts per million (p.p.m.) of selenium from seleniferous wheat indicated that this amino acid was not the answer to the problem. Lewis,

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Schultz, and Gortner (11) concluded from their studies that the addition of 0.45% to 0.89% of DL-methionine to diets containing 25 to 50 p.p.m. of selenite selenium reduced the toxicity of the diets. These same authors reported that cystine gave no protection. In view of this and the postulation by Hofmeister (9) that selenium is at least in part detoxified by methylation and elimination of volatile dimethyl selenide via the breath, they suggested that the protection afforded by methionine resulted not from its sulfur but from its methyl group.

Smith and Stollman (20) found that 0.5% of DL-methionine added to a low protein diet containing 15 p.p.m. of selenite selenium failed to mitigate the toxic effects. On the other hand, Sellers, You, and Lucas (18) reported that DL-methionine showed a protective effect against damage produced by feeding 20 p.p.m. of selenate selenium, but only in the presence of alpha-tocopherol. They found that choline gave no protection. Klug *et al.* (10), however, reported that with diets containing 13 or 19 p.p.m. of wheat selenium, levels of 0.5 to 2.0% of dietary DL-methionine gave no protection whether or not alpha-tocopherol was added.

Methionine has been reported to partially alleviate the toxicity of selenate to yeast (4, 5). Cysteine and gluthathione had no effect. On the other hand, for *E. coli* cysteine and gluthathione had some effect while methionine did not (6).

In view of these conflicting reports, it might well be concluded that the prospect that methionine additions to the diet will prove a practical control measure is poor. However, the identification of dimethyl selenide in the breath of rats injected with selenate (12), and the report that methionine, choline, and betaine containing C_{14} labelled methyl groups and C_{14} formate all supplied to cultures of *S. brevicaulis* on seleniferous media yielded radioactive dimethyl sulfide (3), give further basis to the suggestion of Lewis, Schultz, and Gortner mentioned earlier here. In addition, the report of Rosenfeld and Eppson (17) that the addition of choline to diets increased the rate of growth and duration of life of rats injected with selenium adds strength to the proposal that biologically active methyl groups may reduce the toxicity of the element to some degree.

Studies with Rats

The experiments reported here were undertaken following the report by Baron and Allison (1) that glycocyamine might be essential for optimum utilization of methionine on certain diets. Encouraging results in preliminary trials led to an expansion of the work to include many compounds, and to include experiments with chicks as well as with rats. The work with these different animals will be presented separately for purposes of clarity. (See page 10 for chick studies).

Experimental

The rats used in these experiments were all albino males of the Sprague-Dawley strain. They were placed on experiment at weights of 60 to 80 grams, being individually fed and housed on wire. After about 4 weeks, the survivors were weighed and sacrificed, and their livers were removed and weighed after being blotted dry. The weights of animals that died on experiment were also determined, and their livers were removed for weighing.

Average daily gains were calculated for all rats on experiment, including those that died. This was considered a more accurate measure of growth than gains in weight of survivors only, since the elimination of the rats that died meant rejecting data for the slower gaining animals and giving unrealistically high results for a group where the death rate was high.

For the animals that died, the average daily gain was, of course, calculated only for the period of life of the animal while on experiment.

Liver size, expressed as percent of body weight, is used here as a numerical indication of the extent of liver damage. As reported elsewhere (8) the liver weight:body weight ratio appears to correlate well with visual observation of liver damage. The lower the ratio the greater the damage. Furthermore, its use allows for a more objective measurement of the damage. The most common gross liver defects are atrophy, cirrhosis, and necrosis. The following indicates what various numerical values mean:

Liver Weight (% of body weight)	Average Severity of Symptoms
Above 5.0	Usually no gross symptoms
4.0-5.0	Slight atrophy
2.5-4.0	Moderate to severe atrophy and cirrhosis. Occasional necrosis
Less than 2.5	Usually severe atrophy and cirrhosis. Necrosis common

The diets used in the experimental work varied. They are described in table 1. All seleniferous diets contained 10 p.p.m. of selenium, except in some experiments with the wheat-type diet where other levels were used.

Results

For the purposes of clarity, the work with rats will be presented by diet type rather than in the order in which the various experiments were run.

Wheat-Type Diets: Experiment Ia (table 2) as well as others to be discussed later (under corn-type

Table 1. Basal Diets Used in Rat Studies

Type of Diet	Composition	Non-seleniferous	Seleniferous (10 p.p.m.)	Remarks
Wheat				
	Non-seleniferous wheat (less than 1 p.p.m. Se)	85.5%	35.0%	All additions made at expense of non-seleniferous wheat
	Seleniferous wheat (20 p.p.m. Se)		50.0	
	Casein*	10.0	10.0	
	Brewers' Yeast*	1.0	1.0	Vitamins A and D administered orally once a week (600 IU vitamin A, 85 IU vitamin D).
	Salts (U.S.P. XIV)*	1.0	1.0	
	Lard	3.0	3.0	
Corn				
	Corn	80.9%		Selenium was added to seleniferous diets as sodium selenite to give a level of 10 p.p.m. Se.
	Casein*	12.0		
	Brewers' yeast*	2.0		
	Salts (USP XIV)*	2.0		All other additions made at expense of corn.
	Cottonseed oil	3.0		
	Animal protein factor*	0.1		Vitamins A and D administered orally once a week as above.
Semi-purified				
	Corn starch	71.0%		Selenium was added to seleniferous diets as sodium selenite to give a level of 10 p.p.m. Se.
	Dracket protein†	20.0		
	Salts (USP XIV)*	3.0		
	Solka floc‡	3.0		All other additions made at expense of corn starch.
	Lard	3.0		
	Vitamin mix§	0.14 g/100 g diet		Vitamins A(600 IU), D(84 IU), and E(0.8 mg alphatocopherol) given orally once a week

*Nutritional Biochemicals Corporation

†Purified soybean protein, Dracket Company

‡A cellulose product of Brown Company

§Vitamin mix: thiamine. HCl, 0.6 g; riboflavin, 0.6 g; pyridoxine, 0.6 g; calcium pantothenate, 4.0 g; nicotinic acid, 2.0 g; inositol, 100.0 g; pteroylglutamic acid, 0.2 g; biotin, 0.01 g; vitamin K, 3.0 g; para-aminobenzoic acid, 30.0 g; vitamin B_{12}, .002 g.

Table 2. Effect of Methionine and of Methionine and Glycocyamine on the Toxicity of Seleniferous Wheat Diets

Trial No.	No. of rats per diet	Average initial weight of rats (grams)	Duration of trial (days)	Selenium content of seleniferous diets (p.p.m.)	Addition to basal diets	Data for non-seleniferous diets: Average daily gain in weight (grams)	Average liver size as % of body weight	Survival rate %	Data for seleniferous diets: Average daily gain in weight (grams)	Average liver size as % of body weight	Survival rate %
Ia	5	67	32	10	None	6.1	4.9	100	1.0	3.2	60
					1% glycocyamine+2% DL-methionine	4.3	5.5	100	3.2	6.0	100
Ib	8	68	21	10	None	6.1	5.6	100	0.2	2.8	12
					2% DL-methionine	3.8	5.5	100	1.0*	3.4*	86*
Ic	6	67	28	16	None	5.8	5.2	100	0.2	2.4	67
					2% DL-methionine	3.9	5.9	100	−0.3	3.1	50
Id	7	68	28	11	None	6.6	------	100	0.2*	2.8*	17*
					2% DL-methionine	4.8	------	100	0.5*	3.9*	50*
					2% DL-methionine+1% glycocyamine	4.2	------	100	0.8	3.4	57

*One rat lost from group through accident. Not considered in these results.

diets) stimulated interest in the possibile effect of methyl groups on selenium poisoning. In this experiment, the glycocyamine-methionine addition to the non-seleniferous diets reduced weight gains. On the seleniferous diet, however, weight gains were increased, liver damage was prevented and so were death losses. Although Klug *et al.* (10) had found no beneficial effect from methionine on a similar diet, experiment Ib was undertaken since in their work diets of higher selenium content were used (19 and 13 p.p.m.). This experiment showed methionine itself to reduce weight gains on the non-seleniferous diets. On the seleniferous diets, some slight protection by methionine was evident, but it was not as great as in experiment Ia. In experiment Ic at a higher level of selenium (16 p.p.m.) methionine showed no protective effect, while in experiment Id with diets containing 11 p.p.m. of selenium a slight protective effect was again noted. In this last experiment, glycocyamine did not appear to enhance the effect of the methionine.

One more experiment with seleniferous wheat, using a somewhat modified diet supplemented with vitamins E and B_{12}, was undertaken. In this, DL-methionine, betaine, and choline chloride were added to the diets at about equivalent methyl group levels. The results are given in table 3. Here, the liver:body ratio was somewhat increased in the rats on the methionine diet over that for rats on the seleniferous diet. However, average daily gains and survival were not improved. Betaine gave no protection but choline chloride was slightly effective.

In this series of experiments, the effect of methionine on selenium poisoning was not consistent. It does appear that this amino acid may give some slight protection against selenium poisoning, espe-

Table 3. Effect of Various Compounds on the Toxicity of Modified Wheat-Type Diet*

Addition to Basal Diets	Data for non-seleniferous diets			Data for seleniferous diets		
	Average daily gain in weight (grams)	Average liver size as % of body weight	Survival rate %	Average daily gain in weight (grams)	Average liver size as % of body weight	Survival rate %
None	6.9	6.4	100	1.5	2.8	71
2% DL-methionine	5.1	6.6	100	1.5	4.5	57
0.53% betaine	6.4	6.5	100	1.4	2.8	57
0.63% choline chloride	6.2	6.4	100	2.4	3.2	100

*Basal diet: Wheat 80%, casein 12%, salts U. S. P. XIV 3%, cottonseed oil 3%, brewers' yeast 2%, DL alpha tocopherol acetate 0.05% and vitamin B_{12} 40 p.p.m. Vitamins A and D were administered orally once a week. For seleniferous diets, 50% of seleniferous wheat (10 p.p.m. Se) and 30% non-seleniferous wheat. All additions were made at the expense of non-seleniferous wheat. Average initial weight of rats 70 grams, on experiment 29 days.

cially where levels of selenium fed are lower than those used by Klug *et al.* (10). Since in one experiment, choline chloride also gave some slight protection, it seems possible that methyl groups are involved in some metabolic reaction that will result in decreased toxicity for selenium as it occurs in wheat. The results with betaine do not, however, bear this out.

Corn-Type Diets: The results obtained with the corn-type diet are given in table 4. In experiment IIa, the addition of both glycocyamine and methionine to the diet again gave some protection against the toxicity of selenium. In experiment IIb, while this combination was again effective, methionine alone was effective also, although not to the same extent. Glycocyamine alone had no effect.

In experiment IIc, both the 1% and 2% levels of methionine were effective. Average daily gains were not as good for the 2% level as for the 1% level, but the effect of the methionine itself (as shown by the results for rats on the non-seleniferous diets) probably played a role here. The various glycocyamine-combinations were no more effective than were the two levels of methionine alone.

Glycocyamine alone, at many levels, had no beneficial effects (IId). Another similar experiment with glycocyamine not reported here gave results of a similar nature. Creatine, a metabolic product of methionine and glycocyamine, was also studied (IIe). In this single experiment some slight protection was observed, especially at the 2% level.

Semi-Purified Diets: In the experiments with these diets various levels of several compounds were tested. The basal diet had a calculated methionine content of about 0.2%, which is below that considered optimum. Furthermore, choline was omitted from it. Therefore, the increased rate of gain of the rats on the non-seleniferous diets containing the lower levels of added DL-methionine (table 5) choline or betaine should be expected. At the high levels of these compounds growth rate decreased to below that of the basal alone. L-methionine gave a similar picture, although the highest level used was half that for DL-methionine and the reduction in growth rate was not as severe. Homocystine gradually decreased growth rate with increasing levels of addition.

DL-methionine added to the seleniferous basal increased growth rate, liver:body ratio, and, at the highest level, survival (experiment IIa). It is difficult to evaluate how much of this response is the result of reducing selenium toxicity and how much is the result of merely making the diet more adequate in methionine. The same can be said concerning the responses in average daily gain and liver:body ratio to the L - methionine additions found in experiment IIIb. It appears, however, that methionine may be reducing the selenium toxicity, and the evident response (experiment IIIc) in average daily gain, liver:body ratio, and survival

Table 4. Effect of Various Compounds on Toxicity of Corn Diets Containing Selenite (10 p.p.m. Se)

Trial No.	No. of rats per diet	Average initial weight of rats (grams)	Duration of trial (days)	Addition to basal diets	Data for non-seleniferous diets: Average daily gain in weight (grams)	Data for non-seleniferous diets: Average liver size as % of body weight	Data for non-seleniferous diets: Survival rate %	Data for seleniferous diets: Average daily gain in weight (grams)	Data for seleniferous diets: Average liver size as % of body weight	Data for seleniferous diets: Survival rate %
IIa	4	88	29	None	7.4	5.3	100	3.4	4.6	100
				1% glycocyamine+2% DL-methionine	------	------	------	4.5	6.1	100
IIb	5	67	32	None	6.6	5.6	100	0.9	2.8	20
				1% glycocyamine	5.6	7.5	100	0.9	2.8	20
				2% DL-methionine	4.1	6.4	100	2.5	5.7	100
				1% glycocyamine+2% DL-methionine	4.4	5.4	100	3.9	6.5	100
IIc	8	69	27	None	7.2	5.7	100	0.4	2.3	38
				1% glycocyamine	6.0	7.5	100	0.6	2.7	50
				1% DL-methionine	6.5	5.3	100	4.5	5.8	100
				2% DL-methionine	3.6	5.5	100	2.7	6.3	100
				1% glycocyamine+1% DL-methionine	5.4	5.2	100	4.5	5.6	100
				0.5% glyocyamine+2% DL-methionine	4.0	5.6	100	3.0	6.0	100
				1% glycocyamine+2% DL-methionine	3.7	6.3	100	2.8	6.1	100
				1.5% glycocyamine+2% DL-methionine	2.9	5.9	100	2.6	6.6	100
IId	5	69	28	None	6.7	4.8	100	1.4	3.1	80
				0.025% glycocyamine	7.1	6.1	100	0.5	2.4	20
				0.05% glycocyamine	7.1	5.5	100	0.1	2.3	0
				0.1% glycocyamine	7.3	5.7	100	0.7	2.2	20
				0.25% glycocyamine	6.9	5.7	100	0.3	2.2	20
				0.5% glycocyamine	7.0	7.5	100	0.4	2.3	40
				1.0% glycocyamine	6.4	7.7	100	−0.2*	2.9	0
IIe	6	66	26	None	6.4	5.1	83	1.1	2.9	50
				0.1% creatine	6.3	4.7	100	1.2	2.2	33
				0.5% creatine	6.7	4.7	100	1.0	3.3	50
				1.0% creatine	6.6	5.3	100	1.4	3.2	67
				2.0% creatine	6.6	5.4	100	2.0	3.2	100

*Loss.

Table 5. Effect of Various Compounds on the Toxicity of Semi-purified Diets Containing Selenite (10 p.p.m. Se)

Trial No.	No. of rats per diet	Average initial weight of rats (grams)	Duration of trial (days)	Addition to basal diets	Data for non-seleniferous diets: Average daily gain in weight (grams)	Data for non-seleniferous diets: Average liver size as % of body weight	Data for non-seleniferous diets: Survival rate %	Data for seleniferous diets: Average daily gain in weight (grams)	Data for seleniferous diets: Average liver size as % of body weight	Data for seleniferous diets: Survival rate %
IIIa	8	63	28	None	5.8	6.7	100	0.9	2.2	75
				0.3% DL-methionine	7.1	5.5	100	2.0	2.7	75
				0.8% DL-methionine	6.8	5.5	100	2.4	3.2	75
				1.8% DL-methionine	5.4	5.7	100	1.9	3.1	100
IIIb	7	64	28	None	5.7	5.3	100	0.7	2.7	71
				0.15% L-methionine	6.4	5.6	100	1.1	2.6	71
				0.4% L-methionine	6.5	5.7	100	1.5	2.9	71
				0.9% L-methionine	6.0	5.3	100	2.3	3.3	71
IIIc	8	66	29	None	6.0	5.9	100	0.9	2.8	50
				0.274% homocystine	5.9	6.7	100	0.7	2.4	38
				0.72% homocystine	5.7	7.6	100	1.0	2.6	38
				1.62% homocystine	4.7	6.9	100	1.4	3.8	88
IIId	7	76	28	None	5.9	5.7	100	0.6	3.6	100
				0.1% choline chloride	6.4	6.1	100	0.9	3.9	100
				0.2% choline chloride	6.2	5.7	100	1.5	3.8	100
				0.4% choline chloride	6.2	5.8	100	1.6	4.7	100
				0.8% choline chloride	5.3	5.6	100	1.9	5.5	100
IIIe	8	68	28	None	5.4	5.7	100	0.6	2.4	50
				0.08% betaine	5.7	5.3	100	0.7	2.8	88
				0.17% betaine	5.8	5.6	100	0.7	2.9	75
				0.34% betaine	5.8	5.7	100	0.9	3.5	75
				0.67% betaine	5.3	5.5	100	1.3	3.6	100

to homocystine would seem to add strength to this.

Responses to choline and betaine by rats on the seleniferous diets increased gradually with increasing levels of these compounds to the diet. Again, at least some of these responses could be expected to come from supplying methyl donating compounds to this diet. It should be noted that at levels of choline and betaine where growth on the non-seleniferous diets was suppressed, the greatest average daily gains, liver:body ratios, and (in the case of betaine) survival were obtained on the seleniferous diets.

Studies with Chicks

Single Comb White Leghorn chicks or chicks of Leghorn type were used in all of the studies here reported. Previous work (2) has shown that Leghorn-type chicks are more tolerant of selenium than heavy-type chicks but are less responsive to supplements added to counteract the toxicity. At least two replicate groups of 10 or more male chicks per group were used per treatment in each experiment. The chicks were distributed at random, after wing-banding, into electrically-heated battery brooders. They were given feed and water *ad libitum*. Individual weights were taken at 2 or 3 and 4 weeks of age. The average weights of the replicate groups are presented as a percent of the weight of the control lots grown out in each separate experiment. This made possible more valid comparisons between experiments.

The only symptoms of selenium toxicity which could be noted in these experiments were a reduced rate of growth and a foul odor indicative of the dimethyl selenide being exhaled. Even with 15 p.p.m. of selenium, mortality was very low and that observed showed no relation to selenium toxicity. Therefore only the weight data are here reported.

The formula for the starter diet used for these experiments is given in table 6. This diet as shown is a rather high energy diet averaging 22% protein and approximately 945 Calories of productive energy or 1,430 Calories of metabolizable energy per pound (by calculation). No supplements of the type commonly employed to supply the unidentified factors—i.e. fish meal or

Table 6. Formula of Chick Diet Used

Ingredient	%
Ground Yellow Corn	60
Soybean Meal (50% Protein)	32
Yellow Grease	1.5
Steamed Bonemeal	3
Alfalfa Meal (17% Protein)	2
Limestone	0.5
Salt*	0.5
Vitamin Supplement†	

*Iodized salt containing 2½% $MnSO_4$.
†To supply, per lb., 1800 I.U. Vitamin A, 625 I.C.U. Vitamin D, 2 mg. riboflavin, 2 mg. pantothenic acid, 12 mg. niacin, 52 mg. choline, and 4.5 mcg. Vitamin B_{12}.

Table 7. Methionine and Glycocyamine vs. Selenium Toxicity (Chick Experiments 1 and 2)

	Percent of Control		
	None	0.5% Methionine	1% Methionine
None (Control)	(307)*	112	104
0.5% Glycocyamine	100	106	101
15 p.p.m. Se	66	66	57
Se+Glycocyamine	59	64	65

*Weight in grams at 4 weeks.

fish solubles, dried whey, or meat scraps—were used, since certain animal protein supplements had been shown earlier (14) to exert some protective effects against selenium poisoning. No antibiotic nor vitamin E supplements were used either since some recent unpublished work from this laboratory has suggested that they might also exert some protection against selenium poisoning.

Results

As a result of some earlier studies with chicks and rats, chick experiments 1 and 2 (table 7) were conducted with treatments of 0.5% glycocyamine, methionine at two levels of 0.5 and 1%, selenium at 15 p.p.m. and all of the possible combinations. The first experiment had proved to be so disappointing that the experiment was repeated. Essentially the same results were obtained and are presented here, averaged together for the sake of brevity. In essence, methionine alone had no effect at all on selenium toxicity, whereas the further addition of glycocyamine—in itself somewhat toxic in the presence of single supplements of selenium or methionine—showed a small effect in counteraction of the selenium toxicity. The addition of methionine beyond the 0.5% level is probably also toxic in itself.

Since earlier work with 10 p.p.m. of selenium had shown the combination of gycocyamine and methionine to be more effective than was demonstrated here, it was decided to determine if the level of selenium was of importance. That it is very important was demonstrated by the results of chick experiment 3 shown in table 8. Although the toxicity of 15 p.p.m. of selenium was more severe than that of 10 p.p.m., there was absolutely no effect from the supplements of methionine and glycocyamine on the more acute toxicity. On the less toxic regime, however, the combined supplements were effective in at least partially alleviating the selenium toxicity.

The chick diet used was somewhat deficient in methionine content, in terms of the methionine requirement as an amino acid.

Table 8. Methionine and Glycocyamine vs. Level of Selenium (Chick Experiment 3)

Treatments	% of Control
None (Control)	(282)*
10 p.p.m. Se	72
15 p.p.m. Se	49
10 p.p.m. Se+0.5% Methionine +0.5% Glycocyamine	81
15 p.p.m. Se+0.5% Methionine +0.5% Glycocyamine	49

*Weight in grams at 4 weeks.

However, there is good reason to believe that the reduction in growth rate brought about by the selenium also reduced the amino acid requirement for methionine. It is quite unlikely that the response to methionine on the toxic diets was due to the growth stimulation of methionine *per se*.

To further elucidate the possible role of glycocyamine in this regard, and to determine if other methyl group donors would counteract selenium toxicity, chick experiment 4 was conducted. These results, shown in table 9, would appear to indicate that choline and betaine when used alone were much more effective than methionine in alleviating selenium toxicity. Choline and betaine at the levels of 0.3% contributed a greater proportion of methyl groups than the 0.5% level of methionine. The combinations with glycocyamine showed no great differences in the results obtained with the various methyl group donors, indicating that glycocyamine was effective only in improving the responses to methionine. It is apparent that methyl groups can go only so far in alleviating selenium toxicity. Creatine showed possibly a slight effect.

Table 9. Methionine, Choline, and Betaine plus Glycocyamine vs. Selenium Toxicity
(Chick Experiment 4)

Treatments	Percent of Control	
	None	0.5% Glycocyamine
None (Control)	(264)*	
10 p.p.m. Se	70	60
Se+½% Methionine	77	79
Se+0.3% Choline	86	80
Se+0.3% Betaine	91	81
Se+0.3% Creatine		69

*Weight in grams at 4 weeks.

Table 10. Methionine, Betaine, and Choline vs. Selenium Toxicity
(Chick Experiment 5)

Treatments	% of Control
None (Control)	(307)*
10 p.p.m. Se	73
Se+0.5% Methionine	78
Se+0.3% Betaine	79
Se+0.3% Choline	80

*Weight in grams at 4 weeks.

To determine whether the differences observed between the methyl group donors were real and repeatable, chick experiment 5 was conducted (table 10). Smaller effects were noted for all of the supplements, but the earlier differences were not observed. When used at these levels, these methyl group donors apparently contribute the maximum effective amounts of methyl groups.

It became of interest to determine whether the effect of the methyl group donors in counteracting selenium toxicity were supplementary to that obtainable with arsanilic acid. The results of chick experiment 6 are shown in table 11. Arsanilic acid was used at the 0.04% level since, in the experience of this laboratory (2), that level (being four times higher than recommended for growth promotion) did not improve the growth

Table 11. Betaine and Arsanilic Acid vs. Selenium Toxicity (Chick Experiment 6)

Treatments	% of Control
None (Control)	(275)*
10 p.p.m. Se	76
Se+0.3% Betaine	83
Se+0.3% Betaine+0.04% Arsanilic Acid	83
1 p.p.m. Se	95

*Weight in grams at 4 weeks.

rate of battery fed chicks. Selenium at a non-toxic level was included for academic interest in light of the reports of growth stimulation of rats and chicks with selenium additions to certain purified diets. As the results show, the effects of the methyl group donor, betaine, and arsanilic acid are not additive. Selenium at 1 p.p.m. had no beneficial effect upon the rate of growth. Actually these male chicks grew slower, but the differences are probably not real. Selenium does not appear to be deficient in a practical diet of this type.

Discussion

The results of these studies suggest that methionine may to some degree reduce the chronic toxicity of seleniferous diets to rats and chicks. There is not, however, a great deal more consistency in the findings here than in those previously reported by various authors in the literature, as already discussed.

There is good suggestion from the work on the wheat-type diets (Experiment Ib, Ic, and Id) and with chicks (Experiment 3) that the amino acid has its most apparent effect at the lower selenium levels. While the form of selenium used in the diets may have been somewhat responsible for the variations in results, the type of diet used appears more important in this respect. On the corn-type diet with added selenite, methionine was quite active in reducing toxicity, but on the diets with naturally seleniferous wheat and the semi-purified diet with selenite its effect was considerably less pronounced.

Although work with various levels of added methionine was limited, it appears that the amino acid cannot be expected to give noticeable protection except at rather high levels when it may itself cause a reduced growth rate. Its use as a practical control measure in chronic selenium poisoning does not, therefore, look promising.

Adding glycocyamine along with the methionine did, in some cases, give a slight response with rats and chicks on the seleniferous diet as compared to methionine alone. It is quite possible that the glycocyamine merely reduces the adverse effects of methionine itself in these instances, although the work with chicks indicates its effect to be in improving methionine (or methyl group) utilization.

Choline chloride was somewhat

protective as additions to the naturally seleniferous wheat-type diet and the semi-purified diet containing selenite for rats and to the diet for chicks. Betaine gave no response with rats on the wheat-type diet but did on the semi-purified diet and also gave a response with chicks. It might be said, then, that the apparent small effect of methionine was due to its methyl groups. However, homocystine and creatine also appeared slightly protective. This leaves the role of methyl groups somewhat in question.

Summary

1. Methionine additions to various types of seleniferous diets generally resulted in some protection against toxicity of these diets to rats and chicks. The degree of protection was small and variable, and the results obtained indicate that prospects for the use of this amino acid as a practical control measure for selenium poisoning are poor.
2. Glycocyamine did not appear to consistently increase the effectiveness of the methionine.
3. Choline and betaine also appeared to give some slight protectection with rats and chicks on seleniferous diets. However, similar findings were made with homocystine and creatine, and the work does not clarify the role of methyl groups in the effects observed with the various donors. The further addition of arsanilic acid, although normally effective alone, did not improve the protective effects of betaine.

Literature Cited

1. Baron, H., and Allison, J. B. Effect of methionine and glycocyamine on growth of rats. Fed. Proc., **13**:450. 1954.
2. Carlson, C. W., Guenther, E., Kohlmeyer, Wm., and Olson, O. E. Some effects of selenium, arsenicals, and vitamin B_{12} on chick growth. Poultry Science. **33**:768-774. 1954.
3. Challenger, F., Lisle, D. B., and Dransfield, P. B. Studies on biological methylation. J. Chem. Soc., pp. 1760-1771. 1954.
4. Fels, I. G., and Cheldelin, V. H. Methionine in selenium poisoning. J. Biol. Chem., **176**:819-828. 1948.
5. ———. Biochemical basis of selenate toxicity in yeast. J. Biol. Chem., **185**:803-811. 1950.
6. ———. Reversal of selenate inhibition in *Escherichia coli*. Arch. Biochem., **32**:323-324. 1949.
7. Gortner, R. A., Jr. Chronic selenium poisoning of rats as influenced by dietary protein. J. Nutrition, **19**:105-112. 1940.
8. Halverson, A. W., Hendrick, C. M., and Olson, O. E. Observations on the protective effect

of linseed oil meal and some extracts against selenium poisoning in rats. J. Nutrition, **56**:51-60. 1955.

9. HOFMEISTER, F. Uber methylirung im Thierkorper. Arch. Exper. Path. u. Pharmocol. **33**: 198. 1893-94.
10. KLUG, H. L., HARSHFIELD, R. D., PENGRA, R. M., and MOXON, A. L. Methionine and selenium toxicity. J. Nutrition, **48**:409-420. 1952.
11. LEWIS, H. B., SCHULTZ, J., and GORTNER, R. A., JR. Dietary protein and the toxicity of sodium selenite in the rat. J. Pharmocol. and Exptl. Thera., **68**:292-299. 1940.
12. MCCONNELL, K. P., and PORTMAN, O. W. Excretion of dimethyl selenide by the rat. J. Biol. Chem., **195**:277-282. 1952.
13. MOXON, A. L. Alkali disease or selenium poisoning. S. Dak. Agri. Expt. Sta. Bull. 311. 1937.
14. ———. Some factors influencing the toxicity of selenium. Ph.D. thesis, University of Wisconsin. 1941.
15. MOXON, A. L., and RHIAN, M. Selenium poisoning. Physiol. Rev., **23**:305-337. 1949.
16. ROSENFELD, I., and BEATH, O. A. The influence of protein diets on selenium poisoning. Am. J. Vet. Res., **7**:52-56. 1946.
17. ROSENFELD, I., and EPPSON, H. F. Effect of choline on chronic selenium poisoning of rats. Am. J. Vet. Res., **18**:693-697. 1957.
18. SELLERS, E. A., YOU, R. W., and LUCAS, C. C. Lipotropic agents in liver damage produced by selenium or carbon tetrachloride. Proc. Soc. Expl. Biol. and Med., **75**:118-121. 1950.
19. SMITH, M. I. The influence of diet on the chronic toxicity of selenium. Pub. Health Rpt., **54**: 1441-1453. 1939.
20. SMITH, M. I., and STOHLMAN, E. F. Further observations on the influence of dietary protein on the toxicity of selenium. J. Pharmacol. and Expt. Thera., **70**:270-278. 1941.
21. TRELEASE, S. F., and BEATH, O. A. Selenium. The Chaplain Printers, Burlington, Vt. 1949.

Acknowledgments

These investigations were supported in part by research grant A-813 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Services and a grant-in-aid from Abbott Laboratories, North Chicago, Illinois. This publication was taken in part from a thesis submitted by Eriks Leitis for the degree of Master of Science. The technical assistance of H. R. Woerpel and W. R. Swanson in conducting some of the chick studies was greatly appreciated. Supplies of B-vitamins were kindly donated by Merck & Co. and Chas. Pfizer, Inc., as was DL-methionine by the Dow Chemical Co., and arsanilic acid by Abbott Laboratories.