Immunologic Responses of Mice Fed Diets Supplemented with Selenite Selenium[1] (37391)

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The concept of how selenium (Se) functions in nutritional biochemistry has changed dramatically in the last decade. Initially known for its toxicity when ingested by livestock resulting in "blind staggers" and "alkali disease" (1), Se now is also known to be an essential dietary micronutrient required for normal growth and development and for the prevention of such deficiency diseases as liver necrosis (2), muscular dystrophy (3) and exudative diathesis (4).

Vitamin E has recently been shown to function as an immunoadjuvant, enhancing the primary immune response to sheep red blood cells (SRBC) in chicks (5) and mice (6). Because of the nutritional and biochemical relationship between selenium and vitamin E, experiments were initiated to ascertain if selenium can function as an adjuvant of the primary immune response.

Methods and Materials. Expt 1. Diets in preliminary experiments consisted of Purina chow supplemented with Se as sodium selenite at 0, 0.7, 2.8 or 14–42 ppm. Purina chow is reported to contain ca. 0.5 ppm Se (7).

Expt 2. Diets were formulated from the basal torula yeast diet of Burk *et al.* (8) except that 2.5% of the fat was tocopherol-free corn oil. This diet contains ca. 0.009 ppm Se (7). Selenium supplementation was at 0, 0.75, 1.25, 1.75, 2.25, 2.75, 3.75, 5, 7.5 and 10 ppm. Except for the vitamin E deficient diets, 70 ppm vitamin E was added as *d*-α-tocopherol acetate. Purina chow was also included to compare results with the data obtained in Expt 1.

Swiss Webster weanling mice of both sexes were randomized with no more than 10 animals/cage, fed and watered *ad libitum* and housed at 25°. Mice were fed the experimental diets for 5 wk except for mice fed the Se-toxic diet. These mice received 14 ppm Se for 2 wk and 42 ppm Se for the remaining 3 wk. To survive Se toxicity at 42 ppm, adaptation is required.

After 5 wk all mice were injected ip with 0.2 ml of a 20% suspension of SRBC (ca. 5 $\times$ 10^8 cells) in buffered (pH 7.2) physiological saline. Four days following inoculation with SRBC 4 mice/group were sacrificed. Individual sera were collected and the spleens from two mice were pooled. Seven days following inoculation with SRBC only the sera were collected.

Pooled spleens were macerated by passing through a 400 mesh screen, then diluted with Eagle's minimal essential medium to contain 10^6 cells/ml. The preparation was assayed to determine the relative number of spleen lymphocytes producing antibody (Ab) by the plaque-forming cell (PFC) test (9).

Individual sera were assayed for Ab by hemagglutination of SRBC (10). Sera were also treated with 0.1 *M* mercaptoethanol (ME) to inactivate IgM. IgG is not significantly reduced (11).

Results and Discussion. Results indicate that sodium selenite, like vitamin E, enhances the primary immune response in mice by increasing the number of PFC and the amount of SRBC agglutinating Ab (Figs 1, 2 and 3). Unlike vitamin E which promotes IgG synthesis (6), dietary selenite promotes IgM synthesis.

In Expt 1 the PFC tests showed that mice

[1] Supported by NIH Grant ES 00254-12.

fed the selenium toxic ration had fewer splenic PFC than did the mice fed unsupplemented Purina chow. The number of PFC of mice fed Purina chow diets supplemented with 0.7 or 2.8 ppm Se was only slightly greater than that of control (0 ppm Se) mice. In mice fed the Se toxic ration, the reduced number of PFC was most probably due to Se toxicity.

Mice fed Purina chow diets supplemented with Se at 0.7 and 2.8 ppm had mean Ab titers ca. 7- and 30-fold, respectively, above that obtained from Purina fed controls (Fig. 1). Mice fed Purina chow supplemented with 14–42 ppm Se had a mean Ab titer less than control mice. These results indicate that dietary selenite at levels not toxic to mice can stimulate increased Ab synthesis to the SRBC antigen.

Results from Expt 2 revealed that the relative number of PFC (Fig. 2) increased as the dietary level of selenite was increased from zero to 1.25 ppm. The number of PFC declined, however, as the dietary level of selenite exceeded 1.25 ppm. Nearly a 4-fold increase in PFC from the spleens of mice fed 1.25 ppm Se was observed over that obtained for mice fed Purina chow or the Se deficient diet. Selenite, *per se,* increased the number of PFC when added to the basal torula yeast diet containing 70 ppm vitamin E.

Ab titers of mice fed the torula yeast diet supplemented with vitamin E and Se (Fig. 3) were generally larger than the titers of mice fed unsupplemented torula yeast or Purina chow (Figs. 1 and 3). The Ab titers were not, however, as large as the titers obtained from mice fed Purina chow containing 0.7 and 2.8 ppm Se (Fig. 1). In both experiments the increase in SRBC agglutinating Ab is primarily IgM since little Ab remained following ME treatment.

Mice fed torula yeast diets consumed nearly twice as much food as those fed Purina chow. This inefficiency of food conversion may be responsible in part for limiting synthesis of Ab. Another limiting factor in Ab synthesis may be the deficiency of sulfur amino acids in torula yeast. Antibodies require cysteine to form the cystine linkages between light and heavy amino acid chains. Sulfur amino acid deficiency may additionally account for retarded growth and lowered Ab titers.

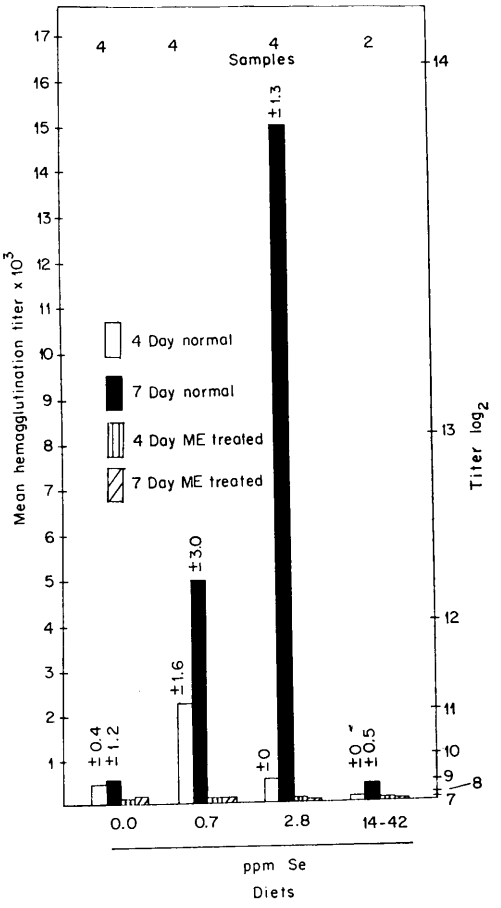

FIG. 1. Antibody titers, as determined by hemagglutination, of mice fed Purina chow diets supplemented with graded levels of selenium. The number at the top of the graph represents the number of mice from a group whose serum Ab was individually assayed. A single determination was made for each serum sample. Titers are shown as mean values ± the standard deviation. Standard deviations are logarithmic deviations from the mean. Logarithmic differences between control and treated are significant ($p > 0.05$). (ME) 2-mercaptoethanol.

Toxicity resulting from selenite ingestion is more noticeable in mice fed torula yeast diets than in mice fed Purina chow. This comparison is reflected in weight gain, spleen weights and mortality as shown in Table I. Highest mortality occurred in mice fed the 5 ppm Se diet with mortality declining in the

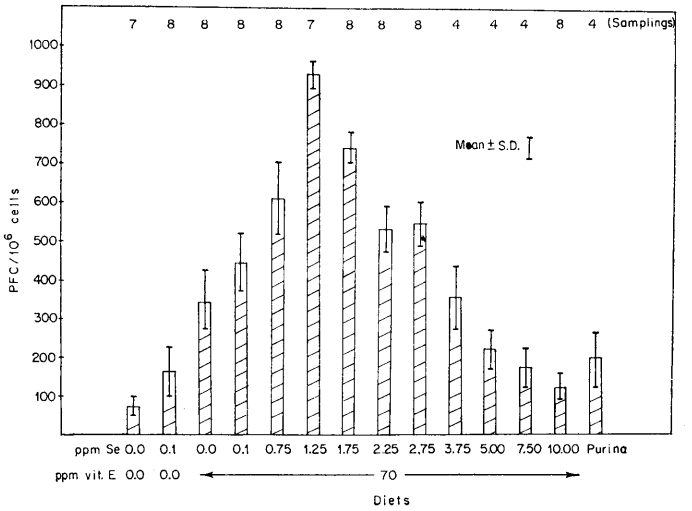

FIG. 2. Four day PFC response of mice. Relative number of spleen lymphocytes producing Ab from the spleens of mice fed semisynthetic diets (torula yeast) supplemented with graded levels of selenium. When possible the PFC test was performed on 4 mice in each group. The spleens from 2 mice were pooled, and from this pool 4 parallel PFC determinations were made. The number at the top of the graph represents the total number of determinations made for each group. Values given are the mean values ± the standard deviation. Differences between "Purina" and "1.25 ppm Se" are significant ($p < 0.05$).

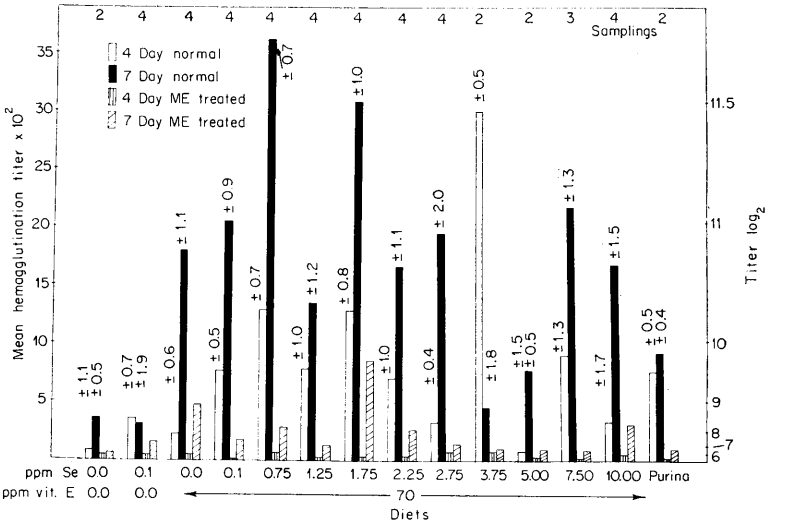

FIG. 3. Antibody titers, as determined by hemagglutination, of mice fed semisynthetic diets (torula yeast) supplemented with graded levels of selenium. The number at the top of the graph represents the number of mice whose serum Ab was individually assayed. Determinations were performed in duplicate on each serum sample. Titers are shown as mean values ± the standard deviation. Standard deviations are logarithmic deviations from the mean. Logarithmic differences between "0 ppm Se–0 vitamin E" or "0.1 ppm Se–0 vitamin E" and "0.75 ppm Se–70 ppm vitamin E" are significant ($p > 0.05$). (ME) 2-mercaptoethanol.

TABLE I. Weight Gain, Spleen Weights and Mortality of Mice in Expts 1 and 2.

Dietary treatment (ppm)	No. of mice	No. surviving	Body wt (av)		Spleen wt (av)	% Body wt
			Start	End		
			Expt 1			
Purina	8	8	9.1	21.6	0.15	0.7
0.7 Se	8	8	6.3	19.6	0.14	0.7
2.8 Se	8	7	6.4	20.4	0.14	0.7
14–42 Se	8	5	7.2	14.5	0.05	0.3
			Expt 2			
Purina	13	12	9.1	24.5	0.18	0.7
—Se—E	28	21	8.9	18.1	0.07	0.4
—Se + 70 E	20	19	9.2	17.6	0.12	0.7
0.1 Se—E	20	19	9.0	20.7	0.13	0.6
0.1 Se + E	20	20	8.9	17.6	0.15	0.9
0.75 Se + E	20	18	8.9	17.4	0.13	0.7
1.25 Se + E	20	13	8.9	22.7	0.12	0.5
1.75 Se + E	20	13	9.6	19.9	0.12	0.6
2.25 Se + E	20	16	9.4	17.8	0.11	0.6
2.75 Se + E	20	12	8.9	18.7	0.10	0.5
3.75 Se + E	20	4	9.4	15.1	0.10	0.7
5.0 Se + E	20	3	9.3	15.0	0.07	0.5
7.5 Se + E	20	5	8.8	19.2	0.11	0.6
10.0 Se + E	20	19	8.6	17.8	0.13	0.7

groups of mice fed the higher Se supplemented diets. The group of mice fed the 5 ppm Se diet had lower Ab titers than Purina fed mice.

The occurrence of greater mortality at 5 ppm Se than at 10 ppm Se is difficult to reconcile. Possibly a third excretory route beyond that of excretion of trimethylselenonium in the urine (12, 13) and dimethyl selenide in the breath (14) may occur when the amount of dietary selenium exceeds 5 ppm. This unusual phenomenon is currently under investigation in our laboratories.

It has recently been demonstrated that injection of coenzymes Q_{10}, (ubiquinone) Q_6 or hexahydrocoenzyme Q_4 stimulates the phagocytic clearance of colloidal carbon from the vascular system of rats (15). In these investigations injection of vitamin E did not increase such clearance. The synthesis of ubiquinone *in vivo* is dependent upon vitamin E, and Se is required for the absorption of vitamin E (16). Previous accounts of the effect of vitamin E on the stimulation of PFC proliferation and humoral Ab synthesis together with the results from this study suggest that the enhancement of PFC and Ab synthesis may in fact occur as a result of increased *in vivo* synthesis of ubiquinone.

Summary. These experiments indicate that dietary Se at levels above that generally accepted as nutritionally adequate (0.1 ppm) enhances the primary immune response in mice as measured by the PFC test and by hemagglutination.

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Received March 2, 1973. P.S.E.B.M., 1973, Vol. 143.