PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 37–40 (1975)

Injectable Selenium: Effect on the Primary Immune Response of Mice[1, 2] (38472)

JULIAN E. SPALLHOLZ,[3] JOHN L. MARTIN, MARLENE L. GERLACH, AND ROLLIN H. HEINZERLING[4]
(Introduced by R. Moreng)

Departments of Biochemistry and Microbiology, Colorado State University, Fort Collins, Colorado 80523

Selenium (Se) is an essential dietary trace element (1) which prevents the Se deficiency diseases, such as liver necrosis (2), exudative diathesis (3) and muscular dystrophy (4) when diets contain ca. 0.05–0.1 ppm Se. Although the precise manner in which Se functions as an essential dietary micronutrient has eluded investigators, it has recently been demonstrated that Se is a component of glutathione peroxidase (EC 11.1.1a) (5) and a cytochrome c-like protein (6). In addition, Se functions in the absorption of lipids, including vitamin E, and is necessary for ubiquinone biosynthesis (7).

We have previously reported that dietary supplementation of Se (ca. 1–3 ppm) as selenite significantly increased anti-sheep red blood cell (SRBC) IgM (8, 9) and IgG (10) antibody (Ab) titers in mice. Both Se deficiency and Se toxicity resulted in Ab titers approximately equal to or less than controls. These experiments were initiated following the reported enhancement of humoral Ab of chicks (11) and mice (12) fed diets supplemented with vitamin E.

The experiments described in this communication were performed in order to ascertain if injectable Se would enhance the primary immune response (PIR) of mice (13, 14) sensitized with SRBC antigen. Additional experiments were performed to determine the effectiveness of Se as an immunoadjuvant when administered to mice prior to, concurrent, or after the SRBC antigen.

Methods and Materials. Female Swiss Webster mice weighing approximately 25 g were used in all experiments. All mice were injected intraperitoneally (ip) with 0.2 ml of a 20% suspension of SRBC (ca. 5×10^8 cells) in buffered (pH 7.2) physiological saline. Individual sera were obtained by brachial bleeding and assayed by the microtiter method (15) for anti-SRBC Ab. In Experiment 2 following the assay of normal sera for anti-SRBC Ab, the sera were treated with 0.1 *M* mercaptoethanol (ME) and reassayed. Treatments with mercaptoethanol predominantly inactivates the anti-SRBC IgM Ab of mice (14, 16).

Experiment 1. In this experiment the effect of 3 or 5 μg Se as selenite or Seletoc[5] on the PIR of mice was studied. Test solutions (0.2 ml) were administered ip simultaneously with the SRBC antigen. Control mice were injected ip with saline (0.2 ml) and SRBC.

Experiment 2. This experiment was designed to determine the effect of the time of administration of Se (5 μg) relative to SRBC sensitization on the PIR of mice.

Groups of mice were: Se injected simultaneously with SRBC antigen (day 0); 1 or 2 days pre-SRBC antigen (day-1, day-2); 1, 2 or 3 days post-SRBC antigen (day +1, day +2, day +3); and simultaneously and for 3 consecutive days post-SRBC antigen (day 0–+3). Control mice were injected with 0.2 ml saline and SRBC as in Experiment 1.

Results and Discussion. Following observations which showed that diets supplemented with Se as selenite enhanced the PIR in mice (8–10), experiments were initiated to ascertain if Se administered by injection would also enhance the PIR of mice to the SRBC antigen (Experiment 1). On day 5 post-

[1] Supported by NIH Grant No. ES 00254-12.

[2] Brief accounts have been reported previously (8, 20).

[3] Present Address: Laboratory of Experimental Metabolic Diseases, Medical Research Programs, Veterans Administration Hospital, Long Beach, CA. 90801.

[4] Department of Dermatology, Henry Ford Hospital, Detroit, Michigan 48202.

[5] Seletoc (courtesy of Burns-Biotec Laboratories, Inc., Oakland, CA 94621; a pharmaceutical preparation containing 1 mg Se as selenite and 50 mg vitamin E as *d*-alpha tocopheryl acid succinate per ml).

SRBC sensitization anti-SRBC Ab titers in mice injected ip with either 3 or 5 μg Se as selenite were significantly ($P > 0.01$) higher than the titers observed for control mice injected with SRBC alone. Seletoc[5] also provided enhanced Ab titers when administered at the 3 or 5 μg Se level (Fig. 1).

These data suggest that Se was the active agent in promoting the enhanced anti-SRBC Ab titers since no significant enhancement of Ab titers was observed when only vitamin E was administered at the 150 or 250 μg level, the dosage of vitamin E administered when Seletoc was used. Berenshtein (17) reported higher anti-tetanus toxoid Ab in rabbits when both vitamin E and Se were injected than when either vitamin E or Se was administered separately.

The results of Experiment 2 are shown in Figs. 2 and 3. Figure 2 shows hemagglutination titers of normal sera and Fig. 3 shows hemagglutination titers of mercaptoethanol treated sera. The results indicate that enhancement of the PIR in mice by selenite alone is most effective when administered prior to or simultaneously with the SRBC antigen. Enhanced titers on day 5, post-SRBC sensitization were observed to occur in mice injected with Se on days 0, −1, −2, and +1 (Fig. 2). Enhanced titers were not observed in mice injected with Se on days +2 or +3 post-SRBC sensitization. In this experiment continuous injections of Se (days 0 to 3) provided the largest titers. Such quantitative differences may not be significant, however, since the doubling time of the IgM Ab is estimated to be only 6 hr (13).

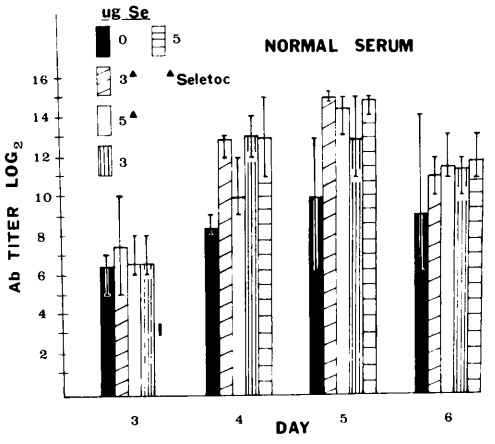

FIG. 1. Anti-SRBC Ab titers of normal sera of mice injected ip with 3 or 5 μg Se as sodium selenite or Seletoc[5]. Titers are shown as mean values and range of four sera individually assayed in duplicate. Logarithmic differences between control and treated mice on day 5 are significant ($P > 0.01$).

Enhancement of anti-SRBC titers of ME treated sera (predominately IgG) commenced between days 6 and 7 post-SRBC sensitization and are most pronounced in the groups of mice receiving Se 1 day prior to (day −1) or concurrent (day 0) with the SRBC antigen (Fig. 3). Titers of mice receiving Se post-SRBC sensitization on day +1, +2, or +3 are not significantly higher than those of control mice.

It is apparent from these data and the report of Berenshtein (17) that Se functions as an immunoadjuvant only when administered prior to or concurrent with an antigen. Furthermore, Se and vitamin E appear to act synergistically in enhancing circulating Ab (18) but only at certain Se Vitamin E concentrations.

Results presented here are similar to data obtained for hemolysin production in mice administered an endotoxin from *Salmonella typhosa* (19). Se, an essential trace element, is also a toxicant. In addition to the possibility that Se may be stimulating the *in vivo* synthesis of ubiquinones (9), Se could also be acting as a cellular toxicant stimulating immunocompetent tissues. The amount of Se, however, which enhances the PIR in mice is not much greater than one day's estimated dietary Se intake. Any hypothesis that Se stimulates immunocompetent tissues does not account for nor would it be in accord with the apparent synergistic effect of Se and vitamin E on the PIR. There appears to be no adverse effect in mice receiving a single dose of Se until about 100 μg Se is administered. There is, therefore, a 20- to 30-fold margin of safety for mice when Se is administered at 3 or 5 μg.

Summary. Sodium selenite administered to mice ip (ca. 5 μg Se) enhances the primary immune response to the sheep red blood cell antigen. Enhancement of the primary immune response is greatest when Se is administered prior to or simultaneously with the sheep red blood cell antigen.

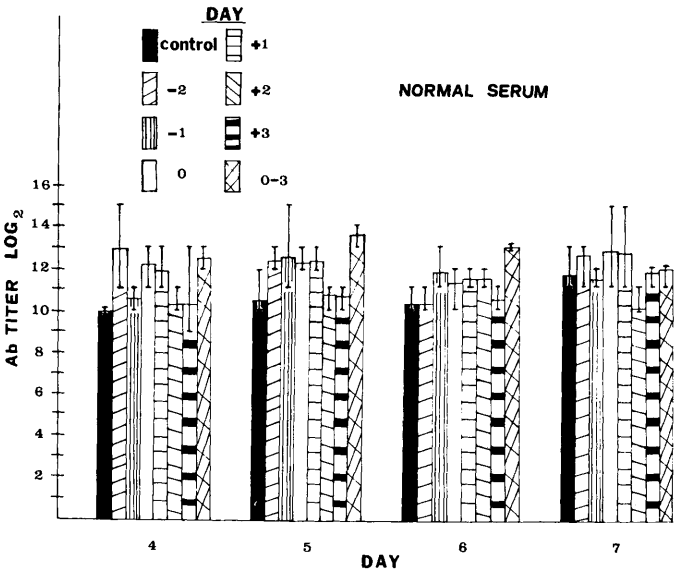

FIG. 2. Anti-SRBC Ab titers of normal sera of mice injected ip with SRBC antigen and saline (contro day 0) or 5 μg Se administered ip 1 (−1) or 2 (−2) days prior to SRBC, simultaneously with SRBC (0), 1 (+1), 2 (+2) and 3 (+3) days after SRBC and for three consecutive days after SRBC (0-3). Titers are shown as mean values and range of four sera individually assayed in duplicate.

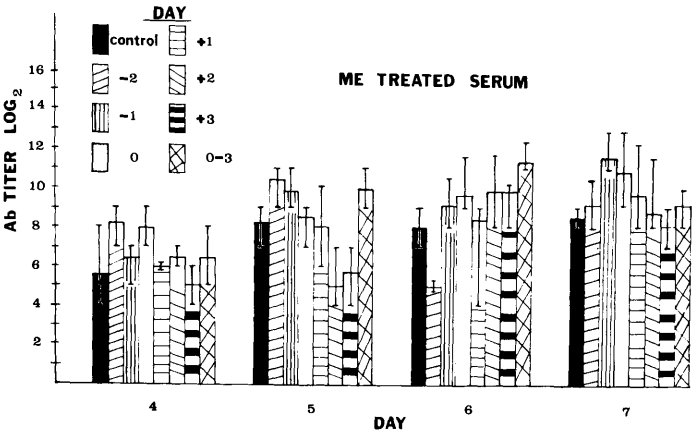

FIG. 3. Anti-SRBC Ab titers of mercaptoethanol treated sera of mice injected ip with SRBC antigen and saline (control, day 0) or 5 μg Se administered ip 1 (−1) or 2 (−2) days prior to SRBC, simultaneously with SRBC (0), 1 (+1), 2 (+2) and 3 (+3) days after SRBC and for three consecutive days after SRBC (0-3). Titers are shown as mean values and range of four sera individually assayed in duplicate.

1. Thompson, J. N., and Scott, M. L., J. Nutrition **97**, 335 (1969).
2. Schwarz, K., and Foltz, C. M., J. Amer. Chem. Soc. **79**, 3292 (1951).
3. Scott, M. L., Hill, F. W., Norris, L. C., Dobson, D. C., and Nelson, T. J., J. Nutrition **56**, 387 (1955).
4. Muth, O. H., Oldfield, J. E., Remmert, L. F., and Schubert, J. R., Science **128**, 1090 (1958).
5. Rotrack, J. T., Pope, A. L., Ganther, H. E., Swanson, H. B., Hoffman, P. G., and Hoekstra, W. G., Science **179**, 588 (1973).
6. Whanger, P. D., Pedersen, N. D., and Weswig, P. H., Proceeding of the Second International Symposium on Trace Element Metabolism in Animals, University Park Press, Baltimore, Maryland and Biochem. Biophys. Res. Commun. **53**, 1031 (1973).

7. Frost, D. V., "CRC Critical Reviews in Toxicology," pp. 467–514 (1972).
8. Spallholz, J. E., Heinzerling, R. H., Gerlach, M. L., and Martin, J. L., Fed. Proc. (Abstract) **32,** 886 (1973).
9. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Proc. Soc. Exp. Biol. Med. **143,** 685 (1973).
10. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Infec. Immunity **8,** 841 (1973).
11. Tengerdy, R. P., Heinzerling, R. H., and Nockels, C. F., Infec. Immunity **5,** 987 (1972).
12. Tengerdy, R. P., Heinzerling, R. H., Brown, G. L., and Mathias, M. M., Int. Arch. Allergy Appl. Immunol. **44,** 221 (1973).
13. Adler, F. L., J. Immunol. **95,** 36 (1965).
14. Adler, F. L., J. Immunol. **95,** 39 (1965).
15. Takatzy, G., Acta. Microbiol. Acad. Sci. Hung. **3,** 191 (1956).
16. Grubb, R., and Swahn, B., Acta. Pathol. Microbiol. Scand. **43,** 305 (1958).
17. Berenshtein, T. F., Zdrovookhr. Belouse. **18,** 34 (1972), and Selenium and Tellurium Abstracts (23317) **14,** 1 (1973).
18. Spallholz, J. E., Heinzerling, R. H., Gerlach, M. L., and Martin, J. L., Federation Proc. (Abstract) **33,** 694 (1974).
19. Franzl, R. E., and McMaster, P. D., J. Exp. Med. **127,** 1087 (1968).
20. Spallholz, J. E., Martin, J. L., Gerlach, M. L., and Heinzerling, R. H., Proceedings of the Second International Symposium on Trace Element Metabolism in Animals, University Park Press, Baltimore, Maryland.

Received April 9, 1974. P.S.E.B.M. 1975, Vol. 148.