and of the protein and peptide content of the granules should provide information about this aging process. Figure 3 shows the two different protein patterns that appear when the contents of both granule fractions are analyzed by slab gel electrophoresis. In the NF-NSG fraction, the smallest protein has a molecular weight of ~ 10,000; in the A-NSG fraction, proteins of 2,000 to 8,000 daltons appear. The peptide content of the A-NSG fraction may also be altered.

The aging of protein-containing secretory granules after maturation (*11*) might have two roles: providing a signal for the eventual lysosomal degradation of granules that are not released, and producing small peptides, whose physiological significance is yet unknown.

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6. The half-life of ^{35}S does not have to be taken into account because the results (Fig. 1) are given as radioactivity of one fraction times 100 divided by the sum of the radioactivity of both fractions. Furthermore, the amounts of hormones and neurophysins in both fractions are very similar (*4*).
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11. In this context, maturation is taken to mean the conversion of a putative precursor into neurophysin and neurohormone. Biochemical studies [H. Gainer *et al.*, in (*2*)] and morphological studies [M. A. Cannata and J. F. Morris, *J. Endocrinol.* **57**, 531 (1973); J. F. Morris and M. A. Cannata, *ibid.*, p. 157] suggest that NSG mature during their axonal transport from the hypothalamus to the neurohypophysis.
12. The normal saline contains 150 m*M* NaCl, 2.2 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 5.6 m*M* $KHCO_3$, and 10 m*M* glucose. The solution was maintained at 37°C and gassed with 5 percent CO_2 in O_2. During depolarization (56 m*M* K^+), the external Na concentration was reduced to maintain tonicity, and control glands were incubated in a medium in which 50 m*M* NaCl was replaced by 50 m*M* choline chloride.
13. The efflux of [^{35}S]cysteine per minute, calculated from the loss of radioactivity, was expressed as a fractional rate constant given by $\Delta x/\Delta t X_t$, where Δx represents counts of ^{35}S released in the time interval Δt and X_t the tissue content of ^{35}S at the midpoint of interval Δt.
14. The gels were made according to the method of R. T. Swank and K. D. Munkres [*Anal. Biochem.* **39**, 462 (1971)], except that the cross-linking agent ethylene diacrylate was used instead of *N,N'*-methylenebisacrylamide. The proteins were obtained after isolation of bovine NSG on an isosmotic gradient (*4*). The granule fractions were washed three times in 0.3*M* buffered sucrose, resuspended in 0.1*N* HCl containing 1 m*M* phenylmethane sulfonylfluoride, 10 n*M* soybean trypsin inhibitor (Sigma), Trasylol (Sigma; 160 kallikrein inhibitor units per milliliter), and frozen. After thawing, the resulting membrane fragments were centrifuged at 35,000*g*; the proteins of the supernatant were separated from the peptides by precipitation with trichloroacetic acid (final concentration, 10 percent). The protein precipitate was dissolved in the presence of 1 percent sodium dodecyl sulfate, 8*M* urea, 1 percent mercaptoethanol, and 0.01*M* H_3PO_4 adjusted to *p*H 6.8 with tris base.
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Lectins of Distinct Specificity in *Rhodnius prolixus* Interact Selectively with *Trypanosoma cruzi*

Abstract. *Lectins of different activities were found in the crop, midgut, and hemolymph of the insect* Rhodnius prolixus. *These were most specific for* N-*acetyl*-D-*mannosamine,* α-N-*acetyl*-D-*galactosamine, and* α- *and* β-D-*galactose, respectively. Lectin receptors were detectable in epimastigote but not in trypomastigote forms of* Trypanosoma cruzi, *a protozoan parasite of the insect and of humans.*

The parasitic protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease in man, is transmitted mainly by hematophagous reduviid insects. The insects become infected by ingesting trypomastigotes from the peripheral blood of infected mammals. In the lumen of the crop and midgut the parasites multiply as epimastigotes, and this multiplication leads to the development of metacyclic trypomastigotes that accumulate in the rectum of the insect (*1*, *2*). In general, *T. cruzi* does not develop in the insect's hemolymph (*3*). The mechanisms underlying differentiation of the parasite and the susceptibility of the insects to infection are largely unknown. Studies of specific recognition mechanisms suggest that host-parasite interactions could be influenced by lectins, a class of sugar-binding proteins of nonimmune origin (*4*, *5*). Lectins interact with cells through their carbohydrate-binding sites, thereby triggering a number of important biologic phenomena (*4*, *5*), such as lymphoblast transformation and cell division, activation of suppressor T cells, and insulin-like stimulation of fat cells; lectins also alter the movement of receptors on cell membranes, producing patching and capping. Membrane-bound lectins appear to play a role in both intercellular and intracellular recognition (*6*). Moreover, lectin receptors are characteristic markers of the developmental stages of *T. cruzi* and of morphologically similar stages from different milieus (*7*). Lectins are usually extracted from plants; they have also been detected in invertebrates, fish, birds, and mammals (*4*, *5*); yet not much is known about their occurrence in insects (*5*, *8*). We now report that lectin activities of distinct carbohydrate specificities are present in the crop (stomach), midgut (intestine), and hemolymph of *Rhodnius prolixus*, an intermediary host of *T. cruzi*, and that each lectin is highly specific in interacting with developmental stages of *T. cruzi*.

Insects were maintained in the laboratory at 25° ± 2°C in a relative humidity of 50 to 60 percent and were fed on human citrated blood every 25 to 30 days. Saliva and salivary glands, crop and crop contents, midgut, rectum, and hemolymph were obtained by procedures described previously (*9*, *10*). Materials were collected from fifth instar larvae or adult insects, from starved animals, or from animals that had had a blood meal 5 days earlier (crop washings were collected immediately after feeding the insects on 0.5*M* NaCl plus 0.001*M* adenosine triphosphate). Salivary glands (50 pairs per milliliter), crop (10 stomachs per milliliter), midgut (10 intestines per milliliter), and rectum (20 insects per milliliter) were homogenized in phosphate-buffered saline (0.1*M* PO_4 and 0.15*M* NaCl, at *p*H 7.2 to 7.4) and extracted in the cold for several hours. The volume of crop washings ranged from 0.1 to 0.2 ml per

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insect. The extracts as well as saliva, crop washings, and hemolymph were centrifuged (770*g* for 60 minutes) at 4°C. The supernatants were passed through Millipore filters (0.22 μm) and assayed for lectin activity. Alternatively, the freshly collected materials were lyophilized and stored for several months, with no detectable change in the original lectin activity.

Hemagglutinating activity was found in the crop, midgut, and hemolymph of *R. prolixus* by screening with erythrocytes from different animals (Table 1). None of the other insect fractions tested, including cultures of the insect's symbiont, *Nocardia rhodnii*, and the insect's feces (100 mg, dry weight, per milliliter), had agglutinating activity. No significant differences in agglutinating activities were found between adult and fifth instar larvae nor between starved and fed animals. Rabbit erythrocytes were agglutinated by all three fractions, but dog erythrocytes were agglutinated only by the hemolymph. With both rabbit and dog erythrocytes, trypsinization strongly enhanced the hemagglutinating power of each active insect fraction. Rabbit erythrocytes treated with sialidase (neuraminidase) were strongly agglutinated by the midgut and hemolymph fractions and to a lesser extent by the crop fraction. The other erythrocytes used (human opossum, sheep, guinea pig, mouse, hamster, and goose) were not agglutinated either before or after the cells were treated with enzymes.

Simple sugars, oligosaccharides, glycoproteins, and lipopolysaccharides inhibited the agglutination of trypsinized rabbit erythrocytes with the same number of agglutinating units of the three active fractions of *R. prolixus* (Table 2). A lectin (or antibody) combining site is considered to be most specific for the hapten that inhibits at the lowest concentration (*5, 11*). Thus, a notable finding was the specificity of the crop hemagglutinin for *N*-acetyl-D-mannosamine (DMan*N*Ac), the best monosaccharide inhibitor. Some other features of the lectin combining site are apparent. The stereochemical arrangement of the acetamido group on carbon 2 of the sugar ring (probably pyranose) is important, since *N*-acetyl-D-galactosamine was inactive as an inhibitor and *N*-acetyl-D-glucosamine inhibited at a concentration 2000 times higher than the minimum required by DMan*N*Ac, an indication that the acetamido group must be in the axial orientation to interact with the combining site of the crop lectin. The importance of the acetyl group is also evident in that D-mannosamine, D-glucosamine, and D-galactosamine were poor inhibitors, approximately 120, 200, and 940 times less active, respectively, than DMan*N*Ac. β-Linked derivatives of D-glucose, such as salicin and sucrose, inhibited at very high concentrations. Glycosides of DMan*N*Ac were not available, but all other saccharides tested were noninhibitors (Table 2). The exquisite specificity of the crop lectin for DMan*N*Ac was further demonstrated when macromolecules were examined as inhibitors, since non-DMan*N*Ac-containing compounds such as blood group substances (hog gastric mucin A + H, human ovarian cyst A, H, B, Lewis[a], Lewis[b], and precursor substances), glycoproteins (fetuin and asialo-fetuin, orosomucoid and asialo-orosomucoid, and bovine thryoglobulin), polysaccharides (streptococcus group A

Table 1. Agglutinating activity of crop washings, midgut extracts, and hemolymph of *R. prolixus* for erythrocytes and for *T. cruzi* epimastigotes. Epimastigotes were from 3-day-old cultures in ox liver-infusion tryptose supplemented with 10 percent fetal calf serum (*7*). Cells were washed five times with phosphate-buffered saline (*p*H 7.2). Equal volumes of trypsin (Difco, 1 mg/ml, *p*H 7.2) or *Clostridium perfringens* sialidase (Sigma type IX, 0.1 unit/ml, *p*H 6.0) and washed cells (8×10^8 per milliliter) were incubated at 37° C for 60 minutes, washed five times with phosphate-buffered saline containing 0.5 percent bovine serum albumin, and resuspended to 1×10^8 to 2×10^8 in the same buffer. Agglutinations were performed with a Takatsy microtitrator (Cooke) in microplates and scored visually with a hand lens and by microscopic observations (*8*). Values are given as the average of three experiments, in agglutinating units per 25 μl of starting materials. One agglutinating unit is the highest dilution of material causing agglutination of the cells.

Source of lectin activity	Treatment of cells	Erythrocytes		*Trypanosoma cruzi* epimastigotes
		Rabbit	Dog	
Crop	None	32	0	0
	Trypsin	512	0	4
	Sialidase	16	0	0
Midgut	None	8	0	16
	Trypsin	128	0	16
	Sialidase	128	0	256
Hemolymph	None	8	4	128
	Trypsin	128	64	0
	Sialidase	128	64	32

Table 2. Inhibition of agglutination of trypsinized rabbit erythrocytes with *R. prolixus* lectins by various carbohydrates and a lipopolysaccharide. The following materials did not inhibit the hemagglutinins at the highest concentration indicated: D-glucose (110 mg/ml); D-mannose (95 mg/ml); D-fructose (50 mg/ml); D-xylose (50 mg/ml); L-arabinose (50 mg/ml); L-fucose (60 mg/ml); L-rhamnose (50 mg/ml); D-fucose (80 mg/ml); trehalose (80 mg/ml); dextran 1355 (5 mg/ml); *Helix pomatica* galactan (2.5 mg/ml); orosomucoid (3.7 mg/ml); asialo-orosomucoid (4.5 mg/ml); fetuin (5.5 mg/ml); asialo-fetuin (2.3 mg/ml); thyroglobulin (2 mg/ml); streptococcus group A and C polysaccharides (2 mg/ml); *Salmonella typhimurium* lipopolysaccharide (1.8 mg/ml); blood group substances A, B, H, Lewis[a], Lewis[b], and precursor substances (1 to 1.2 mg/ml); *N*-acetylneuraminic acid (5 mg/ml); ovomucoid (2.0 mg/ml); bovine submaxillary mucin (1.4 mg/ml); and EDTA (0.2*M*). All sugars were in the pyranose form.

Inhibitor	Minimum concentrations (mg/ml) completely inhibiting 4 hemagglutinating units		
	Crop	Midgut	Hemolymph
N-Acetyl-D-mannosamine	0.1	> 48.0	> 48.0
D-Mannosamine	12.0	> 86.0	> 86.0
D-Glucosamine	20.0	> 80.0	> 80.0
Salicin	42.0	> 42.0	> 42.0
Sucrose	45.0	> 90.0	> 90.0
D-Galactosamine	94.0	> 94.0	> 94.0
N-Acetyl-D-glucosamine	200.0	> 200.0	> 200.0
D-Galactose	> 149.0	> 149.0	5.0
Methyl-α-D-galactoside	> 55.0	0.9	1.8
Methyl-β-D-galactoside	> 47.0	11.5	1.6
p-Nitrophenyl-α-D-galactoside	> 21.0	0.02	2.0
p-Nitrophenyl-β-D-galactoside	> 4.9	0.24	2.4
Melibiose	> 120.0	1.9	4.1
Lactose	> 100.0	50.0	5.8
N-Acetyl-D-galactosamine	> 203.0	0.9	13.0
p-Nitrophenyl-*N*-acetyl-α-D-galactosamide	> 0.5	0.01	> 0.5
p-Nitrophenyl-*N*-acetyl-β-D-galactosamide	> 0.5	0.12	> 0.5
Salmonella weslaco lipopolysaccharide	0.3	> 5.0	> 5.0
Pneumococcus type XIX polysaccharide	1.1	> 4.5	> 4.5

and C, dextrans, mannans), and a lipopolysaccharide (*Salmonella typhimurium*) were all inactive. The only macromolecules that showed high activity were the lipopolysaccharide of *Salmonella weslaco* (*12*) and the pneumococcus polysaccharide type XIX (*13*), which contain DMan*N*Ac. However, not all DMan*N*Ac-containing compounds (for example, pneumococcus polysaccharides type IV, 3.25 mg/ml, and type IX, 4.56 mg/ml) were inhibitors. As far as we know (*4*, *5*) this is the first lectin with specificity for DMan*N*Ac to be reported.

The materials that interacted with the crop lectin were completely inactive toward the hemagglutinins present in the midgut and hemolymph which, in turn, were inhibited by saccharides of the D-galactose (DGal) and *N*-acetyl-D-galactosamine (DGal*N*Ac) configuration. The lectin activities of the latter, however, were quite distinct from one another. Thus, DGal*N*Ac was the best monosaccharide inhibitor of the midgut hemagglutinin, which reacted preferentially with α-linked derivatives of DGal*N*Ac and DGal as compared with the corresponding β-glycosides (Table 2). Melibiose (DGalα1 $\rightarrow$ 6DGlc) was about 25 times more active than lactose (DGalβ1 $\rightarrow$ 4DGlc). D-Galactose did not inhibit at the highest concentration tested. In addition, *p*-nitrophenyl α- and β-glycosides were more than 50 times better as inhibitors, on a molar basis, than the corresponding methyl glycosides; this reflects an apolar interaction between the aglycone part of the glycoside and the corresponding region on the midgut hemagglutinin, as was proposed for other lectins (*14*, *15*). In contrast, the hemagglutinating activity of the hemolymph did not distinguish between the anomers of DGal which, by itself, was somewhat more active than DGal*N*Ac. Methyl and *p*-nitrophenyl glycosides were approximately equal in potency as inhibitors. Additional evidence that the two lectins differ in specificity was provided by their hemagglutination patterns, since the hemolymph agglutinated dog erythrocytes, whereas the midgut extracts did not, either before or after the cells were treated with enzymes (Table 1). Trehalose (DGlcα1 $\leftrightharpoons$ 1αDGlc), the main carbohydrate components of insects, did not inhibit the agglutination by any of the three lectins.

It was of interest to determine whether the *R. prolixus* lectins described above would react with developmental stages of *T. cruzi*, since the lectins are present in the environment where the parasites normally differentiate and multiply. Trypsinized epimastigotes in culture were weakly agglutinated by the crop lectin, whereas the untreated cell, as well as those digested with sialidase, were not agglutinated (Table 1). The midgut lectin, in contrast, agglutinated epimastigotes at high titers, particularly after treatment of the cells with sialidase. A prozone effect was observed in the latter interaction in that the midgut extracts agglutinated the sialidase-treated epimastigotes only after being diluted 8 to 16 times. Trypsinization of the epimastigotes did not significantly affect the activity of the midgut extract. Hemolymph proved to be a very potent reagent in agglutinating epimastigotes, but the activity was abolished and decreased in the trypsin- and sialidase-treated cells, respectively. These findings suggest that crop, midgut, and hemolymph lectins recognize different receptor sites on the epimastigote cell, in accord with their distinct sugar specificity. Agglutinations of the parasite were mediated by the lectin carbohydrate recognition sites, since agglutination was inhibited by the respective specific saccharide haptens at concentrations similar to those that inhibited the erythrocyte agglutination (Table 2). The human serums on which the insects were fed did not agglutinate the parasites. The efficacy of the sialidase treatment on epimastigotes was confirmed by the alteration of the agglutinating pattern of the cells with wheat germ and peanut agglutinins before and after enzyme digestion (*7*).

The interaction of the insect lectins with the trypanosome is stage-specific inasmuch as epimastigotes were agglutinated by the lectins, whereas trypomastigotes isolated from culture medium or purified from the blood of infected mice were not. Similar results were obtained with two strains of *T. cruzi* (Y and Cl). This restricted interaction is further emphasized by the inability of the lectins to agglutinate several other flagellates, namely, *Leishmania donovani*, *L. mexicana amazonensis*, *Tritrichomonas foetus*, *Crithidia deanei* (both normal and aposymbiotic strains), *Herpetomonas samuelpessoai*, and *Phytomonas davidii*. None of these parasites are natural inhabitants of *R. prolixus*. Preliminary experiments indicate the presence of lectin activities in the hemolymph of other blood-sucking insects, such as *Triatoma infestans*, *T. pallidipennis*, *T. vitticeps*, *T. lenti*, *T. matogrossensis*, *Dipetalogaster maximus*, *Panstrongylus megisus*, and *P. herreri*, all highly efficient vectors of *T. cruzi* (*16*).

It was surprising that the midgut and hemolymph lectins did not agglutinate a broad spectrum of cells in view of their reaction with DGal*N*Ac and DGal, two sugars commonly found on cell surfaces. What is more, none of the DGal- and DGal*N*Ac-containing macromolecules that we tested inhibited the agglutination of rabbit erythrocytes and of *T. cruzi* epimastigotes by either lectin, even though these macromolecules, particularly hog gastric mucin A + H substances, are usually strongly reactive with conventional DGal*N*Ac- and DGal-binding lectins (*5*). The restricted agglutinating range of the crop lectin was somewhat expected, since DMan*N*Ac is not as ubiquitous a sugar as DGal*N*Ac and DGal. Nonetheless, the three lectins interacted with the developmental stages of *T. cruzi* in a preferential manner. These considerations suggest a possible biological significance of the lectin-parasite interactions.

Our finding of distinct lectin activities in the crop, midgut, and hemolymph of *R. prolixus* and their selective reaction with *T. cruzi* may provide an approach to study the mechanism of differentiation and multiplication of the parasite in its invertebrate host. The applicability of this system to other invertebrate-parasite complexes should be investigated.

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Oxidation of Elemental Selenium to Selenite by *Bacillus megaterium*

Abstract. *A strain of* Bacillus megaterium *isolated from soil has been found to oxidize elemental selenium in laboratory cultures to selenite and a trace of selenate (< 1 percent of the selenite). This observation represents an important but hitherto unreported oxidative step in the biological selenium cycle.*

This report, the first to our knowledge of a heterotrophic bacterium, *Bacillus megaterium*, oxidizing elemental selenium, provides evidence for the hitherto undocumented microbial oxidation of selenium (*1*). This oxidative step in the selenium cycle is significant in relation to the occurrence and control of the economically important and widespread selenium-responsive diseases of farm animals. According to Muth (*2*), problem areas exist in at least half the states in the United States, and diseases have been recorded in at least 20 other countries, which include most of the major livestock-producing areas of the world. They are of particular importance in New Zealand (*3*).

Microorganisms play an important role in the selenium cycle (*1*), and evidence for the reduction of inorganic forms of selenium is abundant (*1*, *4*). In contrast, the direct microbial oxidation of selenium compounds has received very limited attention. Shrift (*1*) quoted three brief reports of the microbial oxidation of selenium compounds, and in each case selenate is stated to have been produced, from sodium selenite by *Aspergillus niger* (*5*) and from elemental selenium by *Ectothiorhodospira mobile* (*6*) and by a soil bacterium (*7*). However, Bird *et al.* (*5*) did not investigate the oxidation of selenium compounds by molds, and the tabulation indicating selenate as a product of metabolism by *A. niger* with selenite arose from a printing error; "Na_2SeO_4" was misplaced from a position as a column heading to a position in the column under "Na_2SeO_3" (*8*). Lipman and Waksman (*7*) reported the isolation of an autotrophic selenium-oxidizing organism, similar to *Thiobacillus*. This report was later virtually retracted (*9*), and our attempts to isolate an autotrophic selenium-oxidizing organism were unsuccessful. The report on the oxidation of selenium by *E. mobile* (*6*), analogous to the oxidation of sulfur, is similarly without supporting experimental data.

The bacterium was isolated from topsoil from river alluvium, taken to a depth of 7.5 cm, under pasture containing a relatively high concentration of selenium, 1.1 μg/g. The sample was taken at a site in a paddock adjacent to, and 20 km from the mouth of, the Whangaehu River on the North Island of New Zealand. Freshly collected soil was enriched with 0.1 percent of the gray metallic form of elemental selenium and incubated aerobically at 25°C for 6 months. A 1-g sample of soil was then inoculated into 100 ml of growth medium A containing, per liter, yeast extract, 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.3 g; and gray elemental selenium, 1.0 g at *p*H 7.0. Inoculated flasks were incubated at 28°C in an orbital shaker (150 rev/min) for 8 days, and the contents were subcultured (1 : 100 by volume) into medium B (see Fig. 1). Enrichment cultures were incubated for 14 days under similar conditions, centrifuged, filtered (0.45 μm), and analyzed for selenite (*10*), since selenite but not selenate was produced in measurable amounts (selenate < 2 percent of selenite) during the initial 6-month incubation period. The net increase of selenite in these cultures after 14 days was 0.46 μg of selenium per milliliter. Atmo-

Table 1. Oxidation of elemental selenium by *Bacillus megaterium*. Incubation periods with red and gray selenium were 45 and 42 days, respectively.

Treatment*	Selenite production rate† (μg of selenium per liter per day$^{1/2}$)	Selenate produced† (μg of selenium per liter)
Red selenium (0.01 percent) + organism	208 ± 43	13.1 ± 3.5
Red selenium (0.01 percent)	14 ± 4	1.2 ± 0.14
Gray selenium (0.1 percent) + organism	69 ± 10	7.1 ± 0.61
Gray selenium (0.1 percent)	8 ± 3	4.3 ± 1.5

*Four replicates. †Mean ± standard deviation.

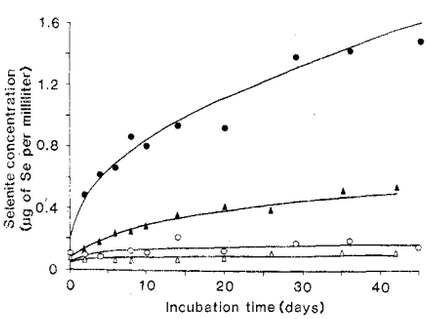

Fig. 1. Production of selenite from elemental selenium by *Bacillus megaterium* isolated from soil. Each experiment was carried out with four replicate flasks, each containing 50 ml of medium B inoculated with 1 ml of a culture 18 hours old (2.2×10^5 cells per milliliter) and incubated at 28°C in an orbital shaker (150 rev/min). Incubations were continued for 45 days (red selenium) and 42 days (gray selenium). Samples were removed periodically and analyzed for selenite (*10*). At the end of the incubation, all samples were centrifuged and filtered (0.22 μm); elemental selenium, selenite, and selenate were separated by anion-exchange chromatography, eluting with 1*M* HCl from a column of Dowex-1 × 8. Total selenium (*12*) was measured in each of 50 fractions per replicate. Medium B contained, per liter, yeast extract, 2.0 g; glucose, 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.30 g; trace elements, 50 ml; elemental selenium, 1.0 g (gray selenium, analytical grade) or 0.1 g (red colloidal selenium); and Tween 80, 0.04 g. The trace element solution contained, per liter, the sodium salt of EDTA (ethylenediaminetetraacetic acid), 5 g; $CuSO_4 \cdot 5H_2O$, 0.02 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; $FeCl_3$, 0.2 g; $Na_2MoO_4 \cdot 2H_2O$, 0.2 g; $ZnSO_4 \cdot 7H_2O$, 0.04 g; and $MnSO_4 \cdot 4H_2O$, 0.4 g. Red selenium was produced in colloidal form dispersed in Tween 80 by reducing selenite with thiosulfate and purifying by dialysis over 7 days. Open symbols designate the control flasks (showing atmospheric oxidation); closed symbols identify the inoculated flasks; triangles, gray selenium; circles, red colloidal selenium. Curves are the best-fit parabolic relations (Table 1). Each data point shown is the mean of four replicates.

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