Form of Selenium in Selenite Enrichment Media for Isolation of Salmonellae

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Selenite-F and selenite-cystine media, commercially available for the routine isolation of salmonellae, were treated by anion exchange chromatography to separate the selenium from other components of the media. A chemical assay, based on an ascorbic acid reduction, showed that the selenium was all in the form of selenite.

The use of selenite to selectively isolate typhoid bacilli, first described by Guth (3), led to the development by Leifson (4) of selenite enrichment media for salmonellae. The precise form of selenium in Selenite-F and selenite-cystine media is of special interest, because salmonellae are one of the few groups of organisms (Astragalus, a genus of higher plants, is another such group) in which so many species show a natural tolerance to selenium, usually a very toxic element (see review, 7, 8). The biochemical basis for this tolerance, though investigated to some extent (5), is incompletely understood.

Because selenite is such a reactive anion, its chemical form in selenite enrichment media is in question. These media contain 0.4% NaHSeO₃ and an undefined mixture of peptones; other substances such as cystine, which apparently enhance the selectivity of selenite (6), are sometimes included. A reaction between selenite and low-molecular-weight thiols is well established (1), and a stoichiometric reaction between selenite and the thiol groups of proteins has also been described (2). Smith (10), on the basis of growth studies with these media, concluded that selenite reacts with and becomes bound to other components of the selenite medium and that the resulting selenium-containing substances, rather than selenite itself, are the ones that inhibit the growth of sensitive organisms. A direct analysis to determine the exact nature of the selenium, however, has not been reported. This note will present evidence that the selenium in both Selenite-F and selenite-cystine media remains as selenite.

Selenite-F and selenite-cystine media were purchased from BioQuest, Cockeysville, Md. Selenite-F medium was prepared without heating, according to the manufacturer's directions. ⁷⁶Se-selenious acid was obtained from Union Carbide Corp., Tuxedo, N.Y., and from Amer- sham/Searle Corp., Arlington Heights, Ill. To remove small amounts of radioactive contaminants, the ⁷⁶Se-selenious acid was purified by anion exchange chromatography (9).

To determine whether the selenium present in the selenite medium was readily precipitable by ascorbic acid, and to determine the amount of such selenium, a selenium assay adapted from an ascorbic acid reduction method (A. A. Tumanov, N. M. Shakhverdi, and Z. I. Glazunova, Chem. Abstr. 67:3731, 1967) was used. To 2-ml samples that contained from 0 to 200 μg of selenium were added 2 ml of 2 N HCl followed by 2 ml of a saturated solution of reduced ascorbic acid. After 5 min, each tube was stirred with a Vortex mixer. Transmission was measured either with a Spectronic-20 at 500 nm or with a Klett-Summerson colorimeter with a blue filter (no. 42). The standard curve with NaHSeO₃ was linear over a range from 0 to 160 μg of Se/ml. The selenium recovered from Selenite-F medium equaled the amount specified by the manufacturer. Unheated selenite-cystine broth gave identical results.

To determine if selenite becomes associated with components of Selenite-F medium, a mixture of ⁷⁶Se-selenious acid and medium was examined by gel filtration chromatography on a Sephadex G-10 column. Materials that absorbed at 280 nm (A₂₈₀) were monitored with a Beckman DB-G spectrophotometer. Radioactivity was monitored with a Packard gamma spectrometer. Figure 1 shows that the peak of radioactivity failed to coincide with any of the A₂₈₀ peaks.

Separation of selenite from A₂₈₀ materials by anion exchange chromatography is illustrated in Fig. 2. Selenite powder was prepared as described above. Much of the A₂₈₀ material was
removed with water; after A_{280} had reached a minimum, HCl of pH 2.0 was applied, and the remainder of the A_{280} material was removed. Radioactivity was eluted by HCl of pH 1.5; it emerged at the position of purified ^{75}\text{Se}-selenious acid. No radioactivity was detected in any of the other fractions. A small rise in A_{280} readings consistently preceded the radioactive peak in each experiment. However, no clear-cut peak accompanied the peak of radioactivity (insert, Fig. 2). A ninhydrin reaction carried out on the radioactive fractions was negative. Identical results were obtained with unheated selenite-cystine medium. Storage of both types of medium in the freezer for several months had no effect on the elution pattern.

To determine how much selenium was actually present in the radioactive fractions, they were pooled, and samples were analyzed for selenite by the ascorbic acid reduction method. The selenium recovered in the pooled fractions accounted for all of the selenium that had been placed on the ion exchange columns.

The ready precipitability by ascorbic acid is one indication that selenium in Selenite-F and selenite-cystine media is still in the form of selenite. Under the acid conditions of this assay, organic trisulfides (R-S-Se-S-R), known to form when sulfhydryl-containing compounds are treated with selenite, would probably be stable (1, 2). The absence of radioactive peaks in association with A_{280} peaks in the Sephadex G-10 and anion exchange eluates, as well as the quantitative recoveries of radioactive selenium in the radioactive peaks, show a failure of selenite to react with components of the media. The anion exchange chromatographic separation of selenite from A_{280} materials and the quantitative recovery of total selenium in the resolved radioactive peaks support this conclusion. Any explanation for the unusual tolerance shown by salmonellae, therefore, must be sought, not in some other form of selenium as has been claimed (10), but in the biochemical properties of selenite.

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LITERATURE CITED
1. Ganther, H. E. 1968. Selenotrisulfides. Formation by the reaction of thiols with selenious acid. Biochemistry 7:2898-2905.
2. Ganther, H. E., and C. Corcoran. 1969. Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. Biochemistry 8:2557-2563.
3. Guth, F. 1916. Selenährboden für die elektive Züchtung von Typhusbacillen. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1. 77:487-496.
4. Leifson, E. 1936. New selenite enrichment media for the isolation of typhoid and paratyphoid (Salmonella) bacilli. Amer. J. Hyg. 24:423-432.
5. McCreary, R. G. L., J. N. Campbell, and J. I. Payne. 1966. Selenite reduction by Salmonella heidelberg. Can. J. Microbiol. 12:703-714.
6. North, W. R., and M. T. Bartram. 1953. The efficiency of
selenite broth of different compositions in the isolation of Salmonella. Appl. Microbiol. 1:130-134.
7. Shrift, A. 1967. Microbial studies with selenium, p. 241-271. In O. H. Muth (ed.), Selenium in biomedicine. Avi Publ. Co., Westport, Conn.
8. Shrift, A. 1969. Aspects of selenium metabolism in higher plants. Annu. Rev. Plant Physiol. 20:475-494.
9. Shrift, A., and T. K. Virupaksha. 1965. Seleno-amino acids in selenium accumulating plants. Biochim. Biophys. Acta 100:65-75.
10. Smith, H. G. 1959. On the nature of the selective action of selenite broth. J. Gen. Microbiol. 21:61-71.