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Possible Origin of Silicon and Tin in the Leahy and NY Post Spore Material

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A puzzling feature of the anthrax letter spores is their high content of silicon and tin relative to what has been reported for any of the numerous other samples of *Bacillus anthracis* analyzed for these elements. It has reasonably been supposed that these features are somehow related to the method by which the letter spores were produced. But the only specific suggestion that has been made in this regard is that, although no such process is known actually to have been applied to bacterial spores, the coats of the anthrax letter spores were deliberately silicone-coated using a tin catalyst in an unspecified but purportedly complex and esoteric process, thereby enhancing their dispersibility (1).

This memo suggests a more mundane explanation of the relatively high silicon and tin levels in the letter spores: the addition of a particular sort of silicon-based antifoam agent to the cultures during bacterial growth and sporulation.

Antifoam agents are commonly added when bacterial growth is done under forced aeration, as in a fermenter, where forced aeration would otherwise generate unacceptable amounts of foam. No such problem arises when cultures are grown in shake flasks or on agar. Several types of antifoam are commonly used in fermenters, some of which are emulsions of silica in polydimethylsiloxane, a silicone oil. Within this class of antifoams, some are produced using organotin as the polymerization catalyst while others are made using organoplatinum or another catalyst.

Although the composition and production methods are generally proprietary, the presence of tin in some antifoams marketed for use in fermenters and its relative absence in other antifoams marketed for such use may be directly shown by mass spectrometric analysis. Table 1 shows the results of such analysis of three silicon-based antifoams currently marketed by Sigma specifically for use in fermenters. All three are described as having a high percentage of "active silicon", ranging between 10 and 30 percent. But only one, Antifoam Y-30, is seen to have a high concentration of tin, about 30 times greater than the other two, antifoams B and SE-15. The foregoing information about the use of tin as a catalyst in the production of some of the several antifoams marketed for use in fermenters, as well as the present analytical results, suggest that the relatively high levels of silicon and tin in the anthrax letter materials resulted from their having been produced by culturing under forced aeration with a tin-containing silicon-based antifoam.

Supposing this suggestion to be correct, a further characteristic of the anthrax letters to be explained is the considerable difference between the bulk contents of different letters with respect to the concentration of silicon and tin. As may be seen in Table 2, the bulk concentrations of these elements in the NY Post letter material are considerably higher than those of the material in the Leahy letter. Nevertheless, energy dispersive X-ray spectroscopy of spores done at Sandia National Laboratory found that the concentration of silicon and of tin in the individual spores of the Leahy and NY Post letters is essentially the same. It is known that

while the material in the Leahy letter is composed almost entirely of spores, the material in the NY Post letter consists of spores together with an approximately ten-fold greater amount of some other material. The higher bulk concentration of silicon and tin in the NY Post material therefore is an attribute not of the spores themselves but rather of the matrix in which they are present.

If, as suggested here, the source of the relatively high levels of silicon and tin in the letter spores is the use during bacterial growth and sporulation of a tin-containing silicon-based antifoam, the greater concentrations of these elements in the bulk material of the NY Post letter indicate that a substantial amount of the antifoam is still present in the NY Post material while the Leahy material, being nearly pure spores, is associated with a much lesser amount of residual antifoam. The considerably lower but still significant concentrations of silicon and tin in ten *B. anthracis* spore batches made at Dugway, noted in Table 2, may also derive from the presence of a silicon-based tin-containing antifoam during culturing, possibly much depleted by washing of the spores or made with an antifoam less rich in tin. Finally, the 12 spore batches made at USAMRIID have only much lower concentrations of silicon and no detectable tin, consistent with the fact that they were produced on agar and therefore without antifoam.

If we provisionally accept the genetic evidence that the letter spores and the spores in flask RMR-1029 derive from the same population, how might the above suggestions affect our thinking about the source of the letter spores? Most of the spores in the flask were produced at Dugway in a 10 liter fermenter in several batches in 1997 all from the same seed stock provided by USAMRIID while some were produced at USAMRIID, probably also from that seed stock. Before addition to the flask, the Dugway preparations were purified at USAMRIID by centrifugation through RenoCal-76 and subsequent washing. Such purification could have removed most of the contaminating silicon and tin from the spores, explaining why spores from the flask had only a low concentration of silicon and no detectable tin. The letter spores, with their high concentrations of silicon and tin, may then have derived from material produced at Dugway that, contrary to a common supposition, was never added to flask RMR-1029 and therefore not subjected to such purification. The high concentration of spores and the absence of detritus in the Leahy letter material indicates, however, that some sort of purification, possibly autolysis and centrifugal washing, was employed in its preparation.

One Dugway batch arriving at USAMRIID was judged to be too impure for successful purification, on the basis of its dark and heterogeneous appearance. Such variation in purity suggests that some sort of preliminary purification, possibly simple washing, was carried out at Dugway before shipment to USAMRIID. The differences between letters in the bulk concentration of silicon and tin and the presence of a *B. subtilis* contaminant in the NY Post material but not in the Leahy material would be consistent with their having come from two different Dugway batches although other possibilities cannot be excluded.

Although the above suggestions are not without forensic implications, their more detailed discussion is beyond the scope of this memo. Meanwhile, there are two experiments that could be done to test the suggested explanations for the relatively high concentrations of silicon and tin in the spore coats: (a) analysis to determine if culturing and sporulation of *B. anthracis* in the presence of tin-containing silicon-based antifoams yields spores with high concentrations of silicon and tin in the spore coats and (b) similar analysis to determine if purification by centrifugation through RenoCal-76 and subsequent washing removes nearly all of these

elements. Also, if spores from one or more of the batches prepared at Dugway in 1997 have been kept, it would be particularly important to analyze them for silicon and tin.

Table 1*
Tin and Nickel in Antifoams Y-30, B AND SE-15

	Y-30	B	SE-15	Y-30/B	Y-30/SE-15
Sn	<u>6,703</u>	250	233	<u>29</u>	<u>27</u>
Ni	4,075	4,160	4,004	0.98	1.02

* Analyses were done by inductively-coupled plasma/mass spectrometry (ICP/MS) at the Harvard Department of Earth and Planetary Sciences. Values are ion counts averaged over three runs. Expression of the results as parts per million requires comparison with standards and is yet to be done. Note that Antifoam Y-30 has ca 30 times more tin than the other two antifoams. The three antifoams did not otherwise differ greatly, as illustrated for nickel and as also shown by the results for 33 additional elements (not shown).

Table 2*
Tin and Silicon in Samples of *B. anthracis* Ames

<u>Sample</u>	<u>Si</u>	<u>Sn</u>
NY Post (Q13) 16 Oct 2002	107,700	6,511
Leahy (Q12) 26 Aug 2002 28 Jun 2004	18,441 14,479	1,979 1,959
Dugway, 10 samples (K1662-K1671) 26 Aug 2002	avg = 7,900 (2,000-50,000)	avg = 86 (33-265**)
USAMRIID 30 Oct 2003 "Burans 12"	avg = 158 (100-265)	not detected

* Data are from FBI Laboratory Reports 020110004 (26 Aug 02); 020605001 (16 Oct 02); 031008001 (30 Oct 03); and 040624018 (28 Jun 04) as provided to the NRC as document B1M7. Analyses were done by inductively-coupled plasma optical emission spectroscopy (ICP/OES). The Dugway samples are from cultures produced in a 10 liter fermenter. **This value and the next highest value (132 ppm) may be inflated owing to possibly poor sample recovery. The average for the remaining 8 samples is 57ppm.

1) Hugh-Jones ME, Rosenberg BH, Jacobsen S (2011) The 2001 Attack Anthrax: Key Observations. *J Bioterr Biodef* S3:001. doi:10.4172/2157-2526.S3-001.