similar. There was a sharp fall in the plasma level and urinary excretion of vitamin C as soon as the subcutaneously injected insulin became effective.

In additional experiments we have found that if glucose is administered intravenously at the time that the plasma vitamin C level and urinary excretion is reduced the effect can be overcome and the plasma level of the vitamin returned to normal.

Similar studies are being made at present on human normal and diabetic subjects.

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Test for the Sterility of Biologic Products.

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Standard tests for the sterility of finished vaccines or serums consist of the inoculation of 0.25 cc and 1.0 cc from the proper number of samples of the product to Smith fermentation-tubes containing infusion-broth. The tubes are heated within 5 hours before inoculation to drive off dissolved oxygen. They are then inoculated and are incubated for 7 days. The presence of weak points in the standard procedure has long been suspected.

The inherent weaknesses of the standard test are, in part, dependent upon (1) the frequent use of merthiolate which, because of its bacteriostatic effect, prevents the growth of contaminants, if any, in many instances; and (2) standard Smith tubes are not well adapted to the cultivation of anaërobes. The use of a second transfer, after 7 days, from optically clear Smith tubes to fresh Smith tubes in an endeavor to overcome the bacteriostatic action of the preservative is definitely irrational and thus unsatisfactory.[1]

The use of Brewer's medium[2] containing thioglycollate overcomes the objections given because it permits the growth of organisms in the presence of merthiolate and because it is suited to the cultivation of anaërobes. The results reported here support these views.

Tests were made with vaccines (bacterins) and serums, preserved with merthiolate, 1:5000 or 1:10,000, or with phenol, 0.5%. As contaminants 24-hour cultures of *Staph. aureus, Staph. albus, Ps. pyocyanea, B. subtilis* and *C. xerosis* were used.[3] In addition, there

[1] Marshall, M. S., and Hrenoff, A. K., *J. Infect. Dis.*, 1937, **61**, 42.

[2] Brewer, J. H., *J. Bact.*, 1940, **39**, 10.

TABLE I.
Growth from Contaminated Serum (1.0 and 0.25 cc Amounts).

	In thioglycollate medium Contaminant		In infusion-broth (Smith tubes) Contaminant	
Preserved with	*Staph. aureus*	*Cl. tetani*	*Staph. aureus*	*Cl. tetani*
Merthiolate, 1:5000	+	+	—	—
" 1:10,000	+	+	—	—
Phenol, 0.5%	+	+	+	—
No preservative	+	+	+	—

were available dried spores of *Cl. tetani* in sand, 10 years old, prepared by Coleman.[4] Vaccines and serums were inoculated lightly with these organisms, singly and in mixtures. From these products 0.25 cc and 1.0 cc respectively, containing from 5 to 500 organisms, were transferred to standard Smith tubes and also to tubes containing 12 cc of thioglycollate medium.* Incubation at 37°C for 7 days followed, with frequent observations.

A summary of results is given in Table I. No difference between results with vaccines and serums was noted. A hundred-fold increase in the inocula did not change the results. *Ps. pyocyanea* appeared to be less sensitive to merthiolate than other organisms; serum preserved with merthiolate, 1:10,000, usually yielded growth and, after several days, serum with merthiolate, 1:5000, usually yielded growth, even in ordinary broth. Dried spores of the *Cl. tetani* failed to develop in Smith tubes in pure culture, although they grew quickly in thioglycollate medium. They failed to develop in Smith tubes in pure culture even from unpreserved serum or serum preserved with phenol. Numerically, an inoculum of 150 spores, the maximum tested, failed to grow in Smith tubes; 10 spores, the lowest number tested, grew readily in thioglycollate medium. In fact, growth of these spores in Smith tubes was observed only when aërobes were also present, and this was irregular.

In summary, it seems clearly evident that the standard test for sterility is inadequate because of the inhibition of growth of possible contaminants by merthiolate, or similar bacteriostatic substances, and because the method is not reliable for anaërobic contaminants. It seems equally clear that thioglycollate medium is adapted to meet these objections and, with suitable refinements, should replace the standard test for sterility.

[3] Rosenstein, C., Levin, I., and Levin, H., *Am. J. Hyg.*, 1935, **21**, 260.

[4] Coleman, G. E., *J. Infect. Dis.*, 1930, **47**, 410.

* Furnished by the Baltimore Biological Laboratories. The formula is: pork infusion from 37.5 g per liter; thiopeptone, 1%; dextrose, 1%; NaCl, 0.5%; Na thioglycollate, 0.1%; agar, 0.05%; methylene blue, 0.0002%.