consistent with the earlier findings of Enders and his coworkers as to the incidence of positive reactors among adult urban populations(6).

A comparison of the agglutination-inhibition and complement fixing titers in 20 mothers on whom both tests were done, showed an independence of the level of antibody as measured by each test. That is, some of the sera having the lowest agglutination-inhibition titers had the highest complement fixing titers, and vice versa. This is in agreement with what Florman and Kutch(4) showed to support the contention that the mumps antibodies measured by the complement fixation and agglutination-inhibition tests were distinct and different.

It cannot actually be determined at the present time whether the mumps viral antibodies are smaller in aggregate size than the streptococcus antibodies. However, if they were, it would offer a possible explanation for some of the greater passive resistance of newborn infants to different viral and bacterial infections.

Summary. Quantitative data are presented to show that both the mumps viral complement fixing and agglutination-inhibiting antibodies are transmitted from mother to child without any significant loss in titer. In contrast, titrations for streptococcus MG agglutinins show in most of the instances at least a 4-fold diminution of the maternal titer in the cord blood. There was no difference in results when specimens were from premature or full term infants.

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Growth of *Actinomyces bovis*: Quantitative Cultural Methods. (18998)

S. B. SALVIN AND B. H. HOYER. (Introduced by C. L. Larson.)
From the Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Mont.

Actinomyces bovis has been grown on a wide variety of media under various cultural conditions. In determining the extent of growth under these cultural conditions, no quantitative technics have been employed. Macroscopic observations with general descriptions of colonial appearance have provided the standards. No methods have been described for using small quantitative inocula. One of the difficulties is that typical rough and intermediate forms(1), when first isolated from parasitic sources in man and animals, appear as non-uniform granules. In addition, many workers transfer undetermined and large numbers of cells with the belief that this technic enhances the possibility of continued growth. Too, the original rough and intermediate forms frequently become smooth after prolonged artificial cultivation.

In order to study the growth requirements of *A. bovis*, it became necessary to develop quantitative methods for determining the size of the inoculum and the amount of growth. Accordingly, experiments were initiated: (a) to preserve the original rough form for an extended period, (b) to obtain maximum growth in liquid media, and (c) to measure the exact amount of growth in various liquid media.

Materials. Three rough isolates from human cases of actinomycosis were used: 43395, which was obtained through the courtesy of Dr. Elizabeth S. Hazen, New York State Department of Health; and AN 11 and AN 14 (from cervico-facial actinomycosis) from Dr. T. Rosebury, College of Physicians and Surgeons, Columbia University. These isolates were grown at pH 7.0 at 37°C in a liquid medium of the following composition: (casitone-yeast extract medium)

casitone (Difco)	15 g
yeast extract (Difco)	5
glucose	5
sodium chloride	2.5
cysteine hydrochloride	.75
sodium thioglycolate	.3
water to make 1000 ml	

1. Rosebury, T., Epps, L. J., and Clark, A. R., *J. Inf. Dis.*, 1944, v74, 131.

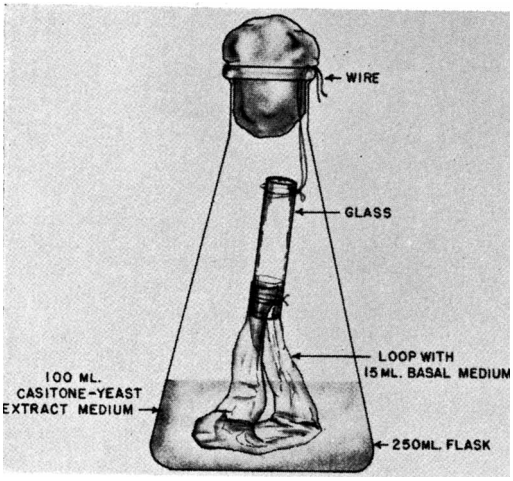

FIG. 1. Diagram of flask with Visking loop for the growth of *A. bovis*.

In the studies employing ridged tubes the casitone and yeast extract were dialyzed before addition to the above medium. Comparable quantities of dialysates were used.

Methods and results. Maintenance of cultures. To maintain the rough form in culture the organism was inoculated into 8-inch loops of Visking tubing containing 15 ml medium suspended in casitone-yeast extract medium (Fig. 1). The original composition of the solution within the Visking loop was sodium chloride, 2.5 g; cysteine hydrochloride, 0.75 g; sodium thioglycolate, 3 g; and water to make 1000 ml (basic salt medium). After 4 to 6 days' growth at 37°C the volume of the internal medium was less than 5 ml, and the growth therein was copious. To insure the availability of the rough forms, the first cultures obtained from the Visking loops were lyophilized.

Determination of standard inoculum. Because of the granular and filamentous nature of the fungus growth, it was impossible to standardize the inoculum by methods based on cell counts. Therefore, 3 to 5 ml of concentrated organisms from a 4- to 6-day-old culture in the Visking tubing were washed thoroughly with 0.85% saline, placed in a 50 ml Erlenmeyer flask with enough 2 mm glass beads to cover the bottom of the flask, and rotated on a Gump shaker for 20 minutes. The resulting suspension was sufficiently homogenous to enable determination of its opacity on a Coleman photo-nephelometer. Serial dilutions of such a cell suspension showed a direct relationship with units on the nephelometer. Measurements were made on the Coleman photo-nephelometer in preference to a photoelectric colorimeter because (a) higher dilutions of the original suspension could be determined directly, and (b) stable reference standards were readily available for instrument calibration. To identify "nephelos units" in terms of concentration of nephelos values of organisms, several different suspensions, prepared as described, were measured and correlated with Kjeldahl nitrogen content (Fig. 2). A direct relationship is indicated between "nephelos units" and amount of cell nitrogen. Therefore, from a number of different suspensions prepared in the manner described above, uniform inocula may be obtained.

Tube culture method for liquid medium. To obtain extensive growth in liquid media, tubes were designed to enable the organism to grow in the optimal zone in relation to the surface when incubated in air. After several trials, the ridged tube, 15 mm × 150 mm (Fig. 3) was found to supply the necessary physical characteristics for growth when inclined at an angle of 30° from the horizontal.

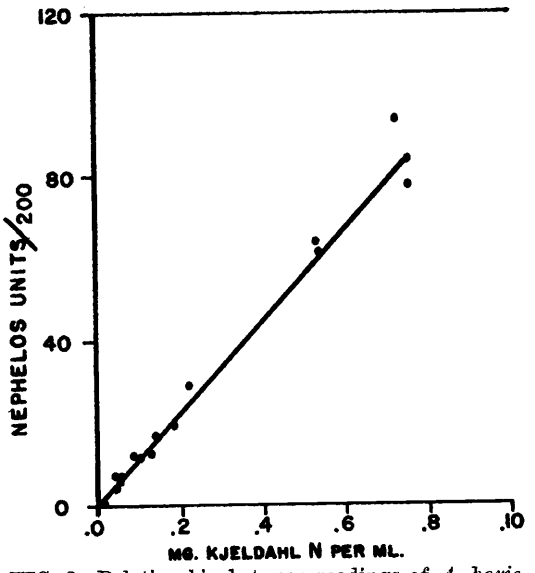

FIG. 2. Relationship between readings of *A. bovis* suspensions (in a Coleman photo-nephelometer) and nitrogen content (Kjeldahl).

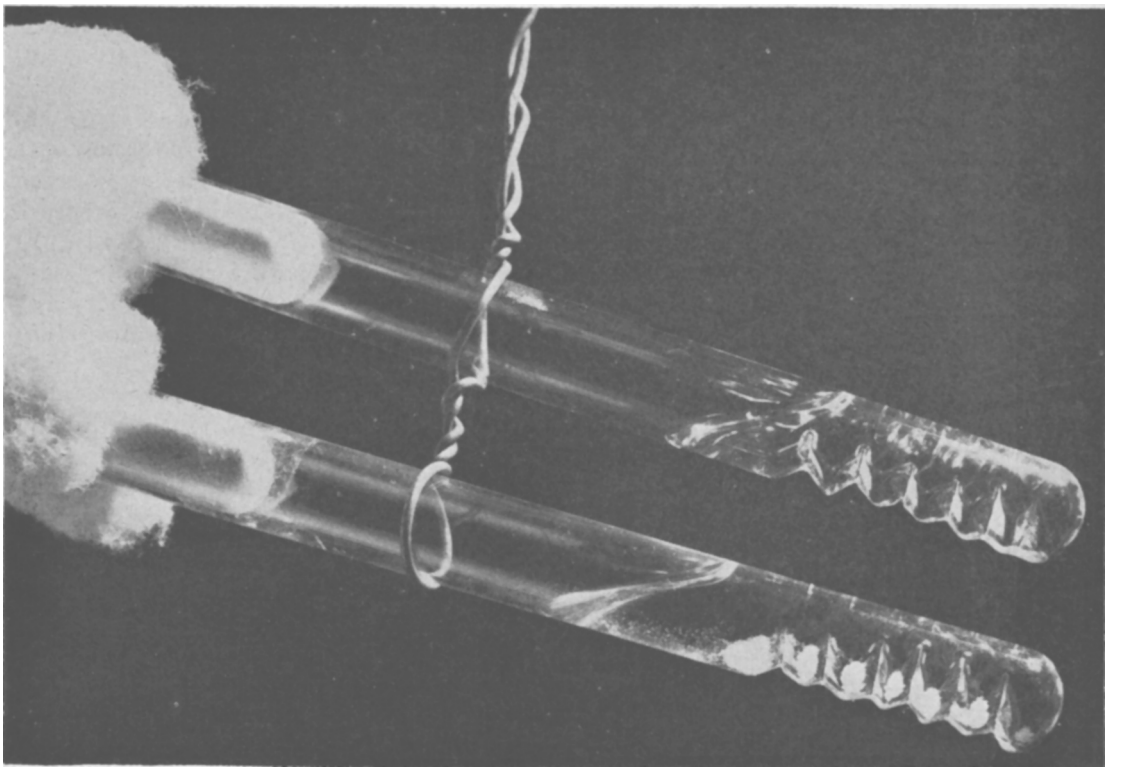

FIG. 3. Photograph of 15 × 150 mm ridged tubes for the growth of *A. bovis*. Upper tube has no growth; lower tube has good growth.

The ridged side, as shown, was placed downward. Plain tubes similarly prepared and inclined showed little or no growth, in comparison with the ridged tubes. In order to insure extensive growth, the ridges were extended to the tube center; shallower ridges gave inferior results. With such ridged tubes, containing 8 ml of the dialyzable medium described, it was found that the minimum standard inoculum for the growth of isolate 43395 corresponded to 0.1 ml of a suspension containing the equivalent of 320 "nephelos units" (equivalent to 1.4 γ Kjeldahl nitrogen). Since the standard suspensions were too concentrated to be measured directly in the nephelometer, appropriate dilutions had to be made to obtain a reading.

Discussion. Growth of *A. bovis* in a liquid medium free of non-dialyzable components provides most advantageous conditions for studies on antigens and growth substances. Also, the use of a liquid medium and the employment of the described methods for accurate determination of the amount of growth offer opportunities for comparison of growth responses under varied experimental conditions.

Summary. (1) Cultural methods are described for obtaining concentrated growth of *A. bovis* in a dialyzable liquid medium. (2) Growth of *A. bovis* has been determined by quantitative technics. (3) A special ridged culture tube has been designed for growing *A. bovis* in a liquid medium under ordinary atmospheric conditions.

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