cooled at 22° C. was concave but not as deeply so as in the case of those cooled at 9° C. The shallowly poured block cooled at 9° C. was solid and of good texture throughout. The shallowly poured blocks cooled at 22° C. and 34° C. were somewhat frothy with small cavities (less than 1 mm. in diameter) throughout the central portion from top to bottom and to within 3 or 4 mm. of each side. The bottom of the shallowly poured block cooled at 9° C. was slightly concave, at 22° C. flat, and at 34° C. slightly convex.

Solid pieces of paraffin shaped from comparable locations in blocks cooled at 9° C., 22° C., and 34° C. were sectioned at 5μ and 10μ. All pieces cut equally well and formed excellent ribbons. Pieces of each ribbon were placed on dry slides as well as on slides smeared with Haupt's adhesive and flooded with 3 per cent formalin water. The wet slides were placed on a warm plate, and after the ribbons had flattened and the water had evaporated, both sets of slides were examined microscopically at 125 and 537 magnifications. No consistent differences in texture, crystal size, or any other characteristic could be perceived or measured.

After establishing an optimum cooling temperature range (22–34° C.), large, medium, and small pieces of plant materials were embedded in boats poured full and medium full of paraffin. When these were cooled at various temperatures within the range, the same kinds of results were obtained as for the solid paraffin blocks.

It is improbable that all types of embedding masses have the same optimum cooling range. For this reason it would seem desirable, especially if unsatisfactory results have been obtained, to establish a range for cooling the particular embedding mass used. For practical purposes the complete optimum temperature range need not be determined. It may be found that very low temperatures resulting from icing the water will produce the best results in some cases. They certainly do not give acceptable results in all cases.

In order to determine the effect of disturbing the cooler bottom layer of paraffin soon after pouring, a boat was poured medium full of paraffin at 56° C., and the bulb of a glass thermometer heated to the same temperature was moved in and over the cooler layer of paraffin in a manner resembling rather rough orientation of materials. Care was taken not to introduce air into the paraffin by lifting and lowering the bulb of the thermometer above the surface. Before crystals began forming on the surface, the thermometer was removed, and the boat of paraffin was cooled within the optimum temperature range. The resulting block was solid but had scattered, minute, low-density areas throughout. This experiment was repeated using a "cold" (room temperature) dissecting needle. The resulting paraffin block contained small holes in the disturbed layer at the bottom of the block and numerous, low-density, pin-point spots throughout much of the block.

Our experiments have shown, therefore, that (*1*) for consistently good results, paper embedding boats should be well filled with paraffin to a depth of ½ inch or more; (*2*) an optimum cooling temperature range should be determined for each type of paraffin embedding mass used; (*3*) rapid cooling of some embedding masses does not appreciably improve the quality of the block to be sectioned; (*4*) the use of cold water for cooling may result in cavitation and low-density areas; (*5*) paraffin blocks with flat bottoms may be obtained by choosing the correct cooling temperature; and (*6*) material orientation maneuvers should be held to a minimum and be performed with instruments warmed to the temperature of the embedding mass.

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The Demonstration of Naturally-occurring Streptomycin-resistant Variants in the Human Strain of Tubercle Bacillus H-37RV[1]

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The development of resistance by tubercle bacilli to streptomycin is of considerable practical importance. To help elucidate this problem, an attempt was made to demonstrate the presence of naturally-occurring resistant variants in a stock laboratory strain of H-37RV by examining a large number of organisms. Variants highly resistant to streptomycin have been demonstrated in cultures of *Shigellae* (*5*), strains of *Staphylococcus aureus*, *Staph. albus*, *Proteus vulgaris*, and *Escherichia coli* (*4*), and *Hemophilus influenzae* (*1*).

TABLE 1

	Streptomycin (μg./cc.)				
	0.74	0.37	0.18	0.09	Control
Growth after 3 weeks	0	0	+++	++++	++++
	0	0	+++	++++	++++

TABLE 2

	Tubes with growth			% showing growth		
	3 weeks	4 weeks	Total	3 weeks	4 weeks	Total
Series I, 100 tubes	3	5	8	3	5	8
Series II, 40 tubes	2	0	2	5	0	5

Cultures were made in the liquid medium described by Dubos (*3*), using 10-cc. rather than 5-cc. quantities. A single 4-week-old culture of the human laboratory strain H-37RV growing in Dubos medium was used as the source of the test organisms. The concentration of tubercle bacilli (mg./cc.) was determined by turbidimetric readings in calibrated tubes, using a blue filter (420 mμ) in a Lumetron colorimeter. Streptomycin assays on the supernatant fluid were done, using *Klebsiella pneumoniae* #41 as the test organism according to the method described by Alture-Werber and Loewe (*2*).

Series I: Each of 100 tubes of Dubos medium containing 18.5 μg. of streptomycin/cc. was inoculated with 0.1 mg. of the test culture of H-37RV.

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Series II: Each of 40 tubes containing 37 μg. of streptomycin/cc. was inoculated with 0.2 mg. of H-37. A routine streptomycin sensitivity assay was done on organisms from the same culture used to inoculate Series I and II with an inoculum of 0.1 mg. Tubes showing growth in Series I and II were centrifuged, the supernatant saved for streptomycin assay, and the bacilli resuspended in fresh Dubos medium. A turbidimetric reading was made and 0.1-mg. amounts were inoculated into tubes for a second streptomycin sensitivity assay.

The assay on H-37 done in duplicate gave 0.37 μg. of streptomycin/cc. as the amount necessary to inhibit growth completely (Table 1). These results agreed with previous testing of this particular strain done in this laboratory. It is apparent that the tubes in Series I contained 50 times the amount of streptomycin necessary to produce complete inhibition of growth, and those in Series II, 100 times the amount.

At the end of 3 weeks, 3 tubes in Series I and 2 tubes in Series II showed definite growth. At the end of 4 weeks, 5 additional tubes in Series I showed growth (see Table 2).

Streptomycin sensitivity assays were done on cultures which showed growth in Series I and II, and the results given in Fig. 1 are representative. The turbidimetric values plotted in the graphs are an average of readings of 4 tubes in each series. Note that the base line for tubes containing 3,700 μg./cc. is at 75. The high concentration of streptomycin gave a distinct yellow color to the medium and resulted in the high initial reading, since the blank tube used for all readings contained only Dubos medium.

Only a fraction of the tubes containing 50–100 times the amount of streptomycin capable of producing complete bacteriostasis on routine sensitivity assay showed growth. The bacilli which multiplied, however, showed a remarkable resistance to streptomycin, being capable of growing in 10,000 times the amount initially adequate to prevent growth. It can be seen in the graph that growth curves for tubes containing up to 1,000 times the amount of streptomycin usually inhibitory parallel the curve for the control tube. If anything, they show a more rapid growth than the controls. Tubes containing 3,700 μg. showed a flatter curve, but growth was quite distinct.

Assays done on the supernatant fluid showed 2 μg./cc. in Series I and 4 μg./cc. in Series II at the end of one month. The tubercle bacilli from these same cultures showed resistance to 3,700 μg./cc. in a subsequent sensitivity assay. Obviously, there had been a gradual loss of streptomycin activity, but even at the end of a month the amount of streptomycin present in Series II was more than 20 times that necessary to inhibit growth in the initial assay.

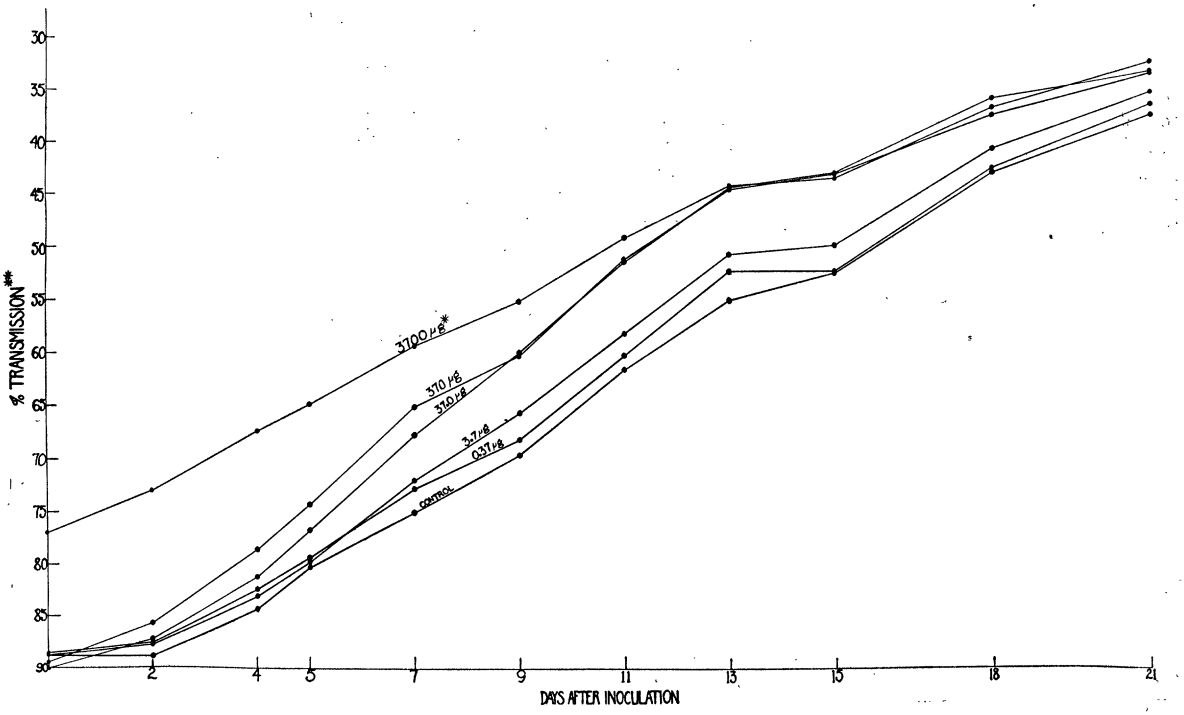

FIG. 1. Streptomycin sensitivity assay on resistant organisms from Series I: * micrograms of streptomycin/cc. of medium; ** % transmission using the blue filter (420 mμ) in a Lumetron colorimeter.

Thus, by using large numbers of bacilli it is possible to demonstrate the occurrence of streptomycin-resistant variants of tubercle bacilli in cultures not previously exposed to streptomycin. It is possible that these naturally resistant variants play a part in the development of streptomycin resistance *in vivo*.

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