

Persistence of Resistance of Tubercle Bacilli to Streptomycin During Passage Through Guinea Pigs.

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Youmans and associates¹ have reported that tubercle bacilli from 8 of 12 patients treated with streptomycin had a resistance to this drug 500 to 1,000 times as great as that of cultures taken before treatment with streptomycin. We have studied cultures from tuberculous patients before and during the course of treatment with streptomycin and also have observed this phenomenon. In some instances cultures of tubercle bacilli have shown a resistance to more than 10,000 times the concentration of streptomycin compared to those obtained prior to treatment.

Preliminary observations indicate that resistance to streptomycin may persist after repeated subculture and storage in the refrigerator. We present herewith our findings that tubercle bacilli isolated from guinea pigs inoculated with sputum, urine or material obtained on gastric lavage from tuberculous patients who were being treated with streptomycin are as resistant as cultures isolated directly from the same materials.

Methods. Specimens of sputum, urine or gastric washings were cultured in 4 tubes of glycerinated egg yolk agar and also inoculated into 2 guinea pigs. Cultures were observed weekly and when growth occurred one tube was selected for determination of resistance to streptomycin. The spleens of the guinea pigs were cultured on the egg yolk agar when the animals died or when they were killed in 8 to 9 weeks. The tests for resistance were done in a manner modified from that used by Youmans and his associates. Two-fold dilutions of streptomycin* ranging from 2,000 to 0.04 μg per milliliter of medium were made in Vorwald's

modification of Proskauer and Beck's medium containing 10% horse plasma. This was dispensed in 5 ml amounts into 150 by 15 mm test tubes. The inoculum was prepared by grinding in a mortar and suspending a portion of the culture in Proskauer and Beck's medium.

The suspension of tubercle bacilli was made to contain approximately 1 mg of bacteria per milliliter as determined by comparison with a standard made by use of the Hopkins tube. Each tube of medium was inoculated with 0.1 ml of suspension or approximately 0.1 mg of tubercle bacilli. We have found that the inoculum may vary within relatively wide limits without any variation being reflected in the results. Duplicate tests of tubercle bacilli with a known resistance to streptomycin have given the same results when 0.2, 0.1, 0.05 or 0.025 mg of inoculum was used. After incubation at 37 C for 14 days the resistance to streptomycin was recorded as the greatest concentration that permitted visible growth. When duplicate tests were made or tests were repeated the results occasionally varied to the next higher or lower concentrations. The apparent large differences as in cases 4 and 12 in Table I are inherent in a 2-fold dilution method.

Results. A comparison of resistance to streptomycin between tubercle bacilli cultured directly from sputum, urine or material removed by gastric lavage and those isolated after passage through guinea pigs is given in Table I. Cultures from untreated tuberculous patients usually are resistant to only 0.08 to 0.62 μg of streptomycin per milliliter of medium and the majority have a resistance of 0.15 μg . Our information is not complete but our present data indicate that resistance of tubercle bacilli from patients being treated with streptomycin is not apparent until treat-

¹ Youmans, G. P., Williston, Elizabeth H., Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clin.*, 1946, **21**, 126.

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TABLE I.
Age of Culture, Duration of Guinea Pig Passage and Resistance to Streptomycin of Tubercle Bacilli Isolated from Patients by Direct Culture and by Guinea Pig Inoculation.

Case	Direct culture		Culture from guinea pig*		
	Age of culture, days	Resistance to streptomycin†	Days in guinea pig	Age of culture, days	Resistance to streptomycin†
1‡	102	0.15	42	60	0.15
2‡	161	0.15	67	94	0.15
3	31	100.0	64	42	100.0
4	24	>2000.0	22	55	500.0
5	114	25.0	71	43	25.0
6	181	25.0	67	114	25.0
7	34	>2000.0	68	30	>2000.0
8	114	10.0	63	50	10.0
9	24	>2000.0	61	37	>2000.0
10	74	0.08	93	32	0.31
11	70	>2000.0	58	33	>2000.0
12	98	1500.0	65	33	>2000.0
13	95	25.0	76	135	25.0
14	112	0.15	60	52	0.15

* Inoculated the same days as direct cultures were made with a portion of the same material.

† Greatest concentration of streptomycin allowing growth in micrograms per milliliter of medium.

‡ Untreated patients.

ment has been carried out for several months.

It is evident from the data presented in Table I that cultures of tubercle bacilli from patients receiving streptomycin retain their resistance to the drug even after residence in guinea pigs for 10 weeks or more and subsequent maintenance on glycerinated egg yolk agar for many weeks.

In another experiment each of 14 guinea pigs was inoculated subcutaneously with 0.1 mg of tubercle bacilli found to be resistant

to at least 2,000 μ g of streptomycin per milliliter of medium. Cultures isolated from 9 of these that died after periods of 61 to 164 days of infection were found still to be resistant to at least 2,000 μ g. In all 14 animals there was gross and microscopic evidence of progressive tuberculosis indistinguishable from that seen in a similar group of guinea pigs inoculated with a sensitive strain from the same patient.

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Uniformity of Size of Bacteriophage Particles.

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As measured by the sintered glass boundary method of Northrop and Anson, Kalmanson and Bronfenbrenner¹ found the apparent diffusion coefficient of coliphage PC (hereafter called T2K) to be about 1.7×10^{-7} cm²/sec, corresponding to an equivalent spherical di-

ameter of about 16 m μ . By fractional ultrafiltration, a very small fraction of the phage was obtained which behaved in the same measurement like particles having a diameter of about 4 m μ .

Immunological analysis² revealed a re-

¹ Kalmanson, G. M., and Bronfenbrenner, J., *J. Gen. Physiol.*, 1939, **23**, 203.

² Hershey, A. D., Kalmanson, G. M., and Bronfenbrenner, J., *J. Immunol.*, 1943, **46**, 281.