IN VIVO DEVELOPMENT OF STREPTOMYCIN RESISTANCE BY THE TUBERCLE BACILLUS*

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THE incidence of the development of streptomycin resistance, *in vivo*, by the *Mycobacterium tuberculosis* has, since the entry of this antibiotic into the therapeutic field been variously reported as ranging over a wide scale.

These reports, far from being conflicting, merely reflect the still widely divergent views and trends associated with dosage and duration of therapy as well as the variety of laboratory methods presently being utilized to arrive at what, at the moment, is no more than an arbitrary conclusion.

The following report is concerned with the development of streptomycin resistance in patients treated at the Toronto Hospital for Tuberculosis, Weston, Ontario. The method used, which is described below, employs a solid medium to which a representative cross section of the bacterial community is transferred following growth in diagnostic Lowenstein's medium. Good growth at a concentration of 50 mcgm. streptomycin per c.c., was taken to indicate resistance, bearing in mind the fact that at least 95% of the cases showed no growth in concentrations of more than 2 mcgm. streptomycin per c.c. prior to therapy.

Methods

Diagnostic culture.—Sputa and fasting gastric were exposed to an equal volume of 23% tribasic-phosphate for 24 hours at a temperature of 37° C. Following centrifuging and neutralizing with 0.1N hydrochloric acid, using bromothymol blue as an indicator, the sediment was planted in two diagnostic Lowenstein's slopes and incubated at 37° C., until positive, or for a maximum of eight weeks. Specimens from genito-urinary cases were, whenever possible, 24 hour urine specimens from which the tannic acid precipitated sediment was similarly treated.

Resistance Tests

A homogeneous suspension of a representative cross section of the growing bacterial community was made by transferring organisms from the Lowenstein's slopes to saline, making certain that samples from as many of the colonies as were apparent were included in the suspension. Equal volumes of the suspension were then spread on a series of Petri dishes containing Herrold's medium.* Each series included a control plate which contained no streptomycin and six others in which the concentrations of streptomycin were as follows: 0.5, 2, 5, 10, 25 and 50 mcgm. streptomycin per c.c. The plates were incubated at 37° C., for four weeks. At the conclusion of this period there was almost invariably confluent growth in the control plate. The growth in the remaining plates were read in degrees of positivity varying from ++++ to 0.

* Herrold's Medium

Peptone	10 gm.
NaCl.	5 gm.
Beef extract (Difco)	3 gm.
Agar	10 gm.
Distilled water to a	1,000 c.c.
pH	7.2 - 7.3

Pour into flasks in 150 c.c. amounts. Autoclave at 15 lb. for 20 minutes. Cool to 56° C. Add streptomycin in the desired amounts. Also at 56° C., add aseptically, one egg yolk to each flask.

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Ratio of resistance to sensitive strains.—While the method described above has the obvious disadvantage that it does not provide the intimacy with streptomycin such as would prevail in a fluid medium, it is however possible, by registering the discrepancy in the degrees of positivity between any given plate and the control, to arrive at a rough estimation of the ratio of sensitive to resistant variants at a given concentration of streptomycin. The arbitrary term "completely resistant" has been applied to those cases in which growth at a concentration of 50 megm. streptomycin per c.c., has approached, visually, the growth at four weeks in the control plate. Further elaboration of this investigation by whatever method, might divulge information which would eliminate those paradoxical problems in which clinical improvement continues despite laboratory assurances that a given case is now "resistant".

TABLE I.

PRE-STREPTOMYCIN SENSITIVITY

Specimen	C	0.5	2	5	10	25	50
Sputum	++ ++	++ ++	++	0	0	0	0

Pre-streptomycin sensitivity tests have been completed in some 225 cases. In 95% of cases results of the order of those shown in Table I have been obtained. In the remaining 5% growth has occurred in 5 megm. streptomycin per c.c., or, very occasionally in a concentration of 10 megm. streptomycin per c.c. It is recognized that the concentrations required completely to inhibit growth are of a higher order than obtains in a fluid media, but comparisons carried out in these laboratories with a modified Dubos medium,* show the discrepancy to be of a constant order of magnitude.

TABLE II.

INCIDENCE OF IN VIVO RESISTANCE

Complete cases (pre- and post-streptomycin cultures)	88	55%
Negative on culture following therapy	72	45%
Total cases	160	100%

Table II indicates the position with regard to 160 cases in which investigations were undertaken before and following therapy. Cases were regarded as being negative when at least one specimen of sputum (three day pool) or one fasting gastric were negative at the conclusion of eight weeks' culture.

TABLE III.

Completely resistant (50 megm.)	37	41.6%
Marked increase in resistance (10 to 25 megm.)	18	20.5%
No significant increase	33	37.7%
Total cases	88	

Table III represents the incidence of streptomycin resistance developing in the 88 cases in which positive cultures were obtained before and after treatment.

Follow-up investigations.—In those cases from which positive cultures may be obtained regularly following therapy, it has been considered of interest to carry out investigations at monthly intervals. In no instance where this has been done in a "resistant" case has it been possible to demonstrate a return to a predominantly sensitive bacterial community, although it is of interest to note that at least on two occasions, resistant variants were not demonstrated until the elapse of some two months following the conclusion of therapy. Continued investigations of this nature are proceeding.

Time of development.—In the majority of cases in which resistance developed, the resistant variants were shown to dominate the cultures at the conclusion of seven to eight weeks of continuous therapy on 1 gm. streptomycin daily. In a relatively small percentage of cases in which resistance was eventually demonstrated, resistant variants did not become apparent for a much longer period and in some instances, already noted, not for several months after the conclusion of therapy.

Direct and indirect methods.—It has been noted, in cases in which tests have been performed by both a direct and indirect method, that in those instances in which the organisms are planted directly to the Herrold's medium without previous incubation and growth in Lowenstein's media, resistance as indicated by growth in serial dilutions seems to be less than if the bacilli were previously grown on artificial media. This may have an explanation in a possible streptomycin content of sputum or alternatively may be related to changes brought about by growth on artificial media.

* Dubos Medium, D.V.A. modification. Personal communications from the Laboratories, Christie Street Hospital, Toronto, Canada.

SUMMARY

(1) The incidence of the *in vivo* development of streptomycin resistance is reported in 88 cases treated by 1 gm. of streptomycin daily in divided doses for periods of 60 to 90 days. (2) attention is drawn to the following points: (*a*) the possible advantage of a solid medium whereby some indication may be obtained of the relative number of resistant and sensitive variants. (*b*) The high incidence of streptomycin sensitive strains encountered prior to therapy. (*c*) The possible delay in the appearance of streptomycin resistant variants even after the conclusion of therapy. (*d*) The absence of a return, even after several months, to a sensitive state. (*e*) The relative early onset of resistance. (*f*) Possible discrepancies in reported results depending on the methods employed.

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