APPRAISAL OF ORAL STREPTOMYCIN AS AN INTESTINAL ANTISEPTIC, WITH OBSERVATIONS ON RAPID DEVELOPMENT OF RESISTANCE OF *E. COLI* TO STREPTOMYCIN*

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THE ACTIVITY OF STREPTOMYCIN against gram negative organisms and its negligible absorption from the alimentary tract, as shown in the writings of Waksman and others,[1, 2, 3, 4, 5] has prompted the oral administration of this antibiotic in an attempt to reduce the bacterial flora in the large bowel preparatory to surgery. It is the purpose of this paper to present an appraisal of the effectiveness of oral streptomycin in reducing the numbers of intestinal bacteria in preoperative cases, in the light of the experiences of this clinic and a review of the publications from other clinics.

Preliminary reports of the effectiveness of streptomycin in intestinal prophylaxis by Zintel[6] were encouraging. In a series of 15 patients who were given 0.25 Gm. of streptomycin by mouth every six hours and followed with quantitative stool cultures, Zintel reported a marked and sustained reduction in the numbers of the three groups of organisms studied (coliform group, *streptococcus fecalis,* and clostridia). The patients in Zintel's series were followed from six to ten days after institution of streptomycin. A later paper[7] reported essentially the same findings in greater detail.

Rowe, *et al,*[8] studied the effects of oral streptomycin and sulfathaladine, singly and in combination, on the coliform group of organisms. These authors gave one group of patients oral streptomycin alone, a second group sulfathaladine, and a third group a combination of the two drugs. The dosage of streptomycin used was 2 Gm. daily. A marked reduction in the numbers of coliform organisms in most of the patients in this series was found to occur within 48 hours, but a reversion was noted in two patients following prolonged administration. These authors concluded that streptomycin should not be used longer than 72 hours preceding surgery.

Poth[9] suggested that streptomycin given orally before surgery might produce deleterious effects by virtue of the marked reduction in the bacterial flora, particularly in the upper alimentary tract, thereby interfering with the absorption of certain fat soluble substances, most important being vitamin K, resulting in an elevated prothrombin time. Another valid objection offered by this author was that in view of the rapid resistance to streptomycin developed by the intestinal bacteria, one would be reducing the usefulness of the drug in the treatment of postoperative complications, should such develop.

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The observations of Paine and Finland[10] on streptomycin resistant and dependent bacteria are pertinent in this connection. While our work was in progress these authors reported work along similar lines with various groups of organisms, including *E. Coli.* They report that strains of streptomycin-sensitive, streptomycin-resistant, and even streptomycin-dependent organisms appear after exposure to various concentrations of streptomycin.

A series of 24 cases receiving oral streptomycin alone and nine cases receiving streptomycin and sulfathaladine in combination have been studied with quantitative estimations being made of the changes in stool bacteria which occur during preoperative administration of these drugs. The cases reported were selected at random from among patients admitted to Presbyterian Hospital for large bowel lesions; the diagnoses included carcinoma of the rectum, carcinoma of the sigmoid, chronic ulcerative colitis, diverticulitis, polyposis, and fistula in ano (tuberculous and non tuberculous). In this series, the three major groups of alimentary tract organisms were studied, namely the coliform group, the intestinal streptococci, and the clostridia, by the method described below.

BACTERIOLOGIC TECHNIC

Stools were obtained for culture before and every one or two days after the institution of oral streptomycin. The cases were followed for periods ranging from five to 14 days after the institution of chemotherapy. An emulsion of 1 Gm. of fresh wet stool in 9 cc. of sterile broth was prepared in a glass plunger type homogenizer. This 1:10 dilution of the stool was then carried through a continued series of eight 1:10 dilutions so that the last was $10^{-8}/x$ the concentration of the original stool specimen. One-half cubic centimeter portions of tubes two, four, six and eight were used for inoculating agar plates of the following media:

(1) Eosin methylene blue for coliform organisms. Surface lactose-fermenting colonies were counted after 24 hours incubation at 37° C.

(2) Modified Wilson Blair for clostridia. Two-tenths cubic centimeter of 8 per cent Ferric Chloride and 2.0 cc. of 20 per cent sodium sulfite were added just before use to 20 cc. of liquid meat infusion agar. Pour plates were made, the colonies of clostridia appearing as black spots in the medium after 24 hours anaerobic incubation.

(3) Two per cent Dextrose meat infusion agar, buffered with NaOH to pH 9.3 for streptococci. Pour plates were also made with this medium, the streptococci appearing as small millet seed shaped colonies in the medium, and colonies were counted after 72 hours incubation.

Colony counts were then made on the plate of highest dilution in which colonies appeared for each of the three groups of organisms. By this method a relatively accurate approximation of the total number of organisms in each stool was gained.

RESULTS AND INTERPRETATION

A total of 24 patients received oral streptomycin alone. 19 of whom received

0.25 Gm. every six hours, the other five receiving 1.0 Gm. every six hours. No essential difference in results obtained was noted in the two groups.

The effects of 0.25 Gm. of oral streptomycin upon the three classes of organisms studied in the group of 19 preoperative patients scheduled for large bowel surgery receiving the dosage are shown in the accompanying table (Table I). The discrepancy in the total number of patients upon whom counts were done for the three types of organisms is due to the fact that technical errors in preparing media rendered two streptococcus controls

TABLE I.—*Response of Colony Counts to 1.0 Gm. of Streptomycin Per Day*

Degree of Response	Number Giving No Response	Number Giving Temporary Response		Number Giving Prolonged Response		Total Number of Cases
		To 0.01%	To 0.001% or Less	To 0.01%	To 0.001% or Less	
E. Coli	9	1	4	1	4	19
Streptococcus	15	1	0	0	1	17
Cl. welchii	7	0	4	0	7	18

and one *clostridium welchii* control unreliable. These and subsequent cultures on the same patients were therefore not included in the results tabulated.

The patients were grouped according to whether they gave (1) no significant response to the drug (*i.e.*, a drop to not less than 0.1 per cent of the original colony count), (2) a temporary response, and (3) a prolonged response. These two latter groups were subdivided into (a) those which dropped to 0.01 per cent of the original count and those which dropped to 0.001 per cent of the original count or less. To allow for the expected variation in the method no drop in colony count of less than to 0.01 per cent of the original count was considered significant in terms of clinical benefit to be derived although a drop to 0.1 per cent of the initial value is probably bacteriologically significant. In all instances in which the reduction in colony count was to 0.001 per cent of the original count or less, the remaining population was 200 organisms or less per gram of wet stool, this being the smallest number which could be counted by this technic.

Of the 19 patients receiving 1 Gm. of streptomycin per day, nine, or 47.7 per cent, showed no significant response in the *E. Coli* colony counts, and 10, or 52.3 per cent, showed a significant response. Of the latter group only five (26.1 per cent) remained at a lowered count for the duration of the preoperative period. In these five cases the last stool cultures were obtained five, seven, eight, 13, and 14 days after the institution of oral streptomycin. In all ten cases in which there was a significant drop in colony count, this had occurred by the second day in seven patients, by the third day in two, and by the fourth day in one patient. This would indicate that in those which do respond the maximum response is obtained by the second to the fourth day on this regimen, after which a large proportion of the responsive cases (50

per cent in this series) will show a rapid return to the original bacterial counts. (In the light of these observations an investigation of the development of resistance by various strains of *E. Coli* to streptomycin was undertaken. This will be discussed in more detail below.)

Among the 17 patients upon whom serial counts were done for streptococci, 15, or 87.7 per cent, showed no significant reduction in the number of organisms, one showed a temporary reduction, and one a prolonged reduction. This would suggest that very little reduction in streptococcal population may be expected by the oral administration of streptomycin.

Among the 18 patients in whom counts were carried out for clostridia, seven, or 38.9 per cent, showed no significant reduction in the total count; four, or 22.2 per cent, a temporary reduction, and seven, or 38.9 per cent, a prolonged reduction. The reduction in the number of clostridia present generally occurred more gradually than with the coliform group, when a drop did occur, the decline taking place between the second and the seventh day. The subsequent rise, when it occurred, took place within two to four days after the maximum reduction.

Five cases received 4 Gm. of streptomycin per day (1 Gm. every six hours). A tabulation of these figures (Table II) shows that there appears

TABLE II.—*Response of Colony Counts to 4.0 Gm. of Streptomycin Per Day*

Degree of Response	Number Giving No Response	Number Giving Temporary Response		Number Giving Prolonged Response		Total Number of Cases
		To 0.01%	To 0.001% or Less	To 0.01%	To 0.001% or Less	
E. Coli	3	0	0	0	2	5
Streptococcus	4	0	0	0	1	5
Cl. welchii	2	0	1	0	2	5

to be no significant difference between this small group of cases and those treated with 1 Gm. per day. Because of the rapid development of resistant strains of organisms and the lack of significant difference in results, the larger dosage was abandoned in our series of cases.

None of the toxic effects seen with the parenteral administration of streptomycin were observed in any of the series of cases. Prothrombin times were determined by the Quick method every two to four days in 11 patients in this series and in seven of them there appeared a slight prolongation. In no instance was this increase more than 4.5 seconds, 22 seconds being the highest prothrombin time recorded in this group (14 ± 1 second being normal). No consistent correlation was found between a rise in prothrombin time and a drop in bacterial flora.

DEVELOPMENT OF RESISTANCE OF E. COLI TO STREPTOMYCIN

The rapid return to pre-treatment levels of bacterial counts in so many of our patients led to an investigation of the development of streptomycin

resistance by *E. Coli* in a group of ten cases chosen at random from this series.

Preliminary titrations had shown that in these patients the *E. Coli* isolated before administration of oral streptomycin were sensitive to and completely inhibited by 1.25 units of streptomycin per cubic centimeter. After treatment the organisms rapidly became resistant to at least 100 units/cc. Therefore a wide range was selected, *i.e.* 0 to 10,000 units/cc., distributed as in Figure 1.

STREPTOMYCIN RESISTANCE OF E. COLI

UNITS/C C.		0	19	39	78	156	312	625	1250	2,500	5,000	10,000
LO.	0	+	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	o	o	+	+	+
	4	+	+	+	+	+	+	o	o	+	+	+
CO.	0	+	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	o	o	+	+	+
	4	+	+	+	+	+	+	o	o	+	+	+
BO.	0	+	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	o	o	+	+	+
	4	+	+	+	+	+	+	o	o	+	+	+
O'D.	0	+	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+
EP.	0	+	o	o	o	o	o	o	o	o	o	o
	3	+	+	+	+	+	+	o	o	+	+	+
	5	+	+	+	+	+	+	o	o	+	+	+
TR.	0	+	o	o	o	o	o	o	o	o	o	o
	3	+	o	o	o	o	o	o	o	o	o	o
	5	+	o	o	o	o	o	o	o	o	o	o
	6	+	+	+	+	+	+	+	+	+	+	+
BR.	0	+	o	o	o	o	o	o	o	o	o	o
	4	+	+	+	+	+	o	o	o	+	+	+
CR.	0	+	+	+	+	+	o	o	o	+	+	+
	2	+	+	+	+	+	o	o	o	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	o	o	o	+	+	+
RI.	0	+	o	o	o	o	o	o	o	o	o	o
	8	+	+	+	+	+	+	o	o	+	+	+
PU.	0	+	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	+	o	+	+	+
	4	+	+	+	+	+	+	o	o	+	+	+
	6	+	+	+	+	+	+	o	o	+	+	+
	8	+	+	+	+	+	+	o	o	+	+	+

+ INDICATES GROWTH AT GIVEN STREPTOMYCIN CONCENTRATION
0,2,4 INDICATES DAY OF THERAPY WHEN CULTURE WAS OBTAINED

FIG. 1

From each stool specimen the *E. Coli* were isolated in pure culture on EMB agar. An inoculum for titration was prepared by placing one loop of the organism into five cubic centimeters of streptomycin broth (a standard specified by the U.S.F.D.A.) and incubated at 37° C. for five hours. One drop of this inoculum was added to two cubic centimeters of the titration broth and incubated for 72 hours at 37° C. and read at the end of that time for visible turbidity.

The results revealed that prior to treatment the *E. Coli* were inhibited in all but one case by the lowest concentration of streptomycin used, *i.e.*, 19

units/cc. This one exception was resistant to 156 units/cc. After 48 hours of oral streptomycin, the organisms isolated were found to be resistant to at least 156 units/cc. in all but one case, this strain requiring six days for the development of resistance. In one case the organism grew in all the titration concentrations after 48 hours of oral streptomycin. This was observed on prolonged treatment in two other cases.

A marked prozone phenomenon was observed in these titrations. In the 2500 units/cc. concentration and up flocculation was produced which obscured the gross reading by turbidity, so that growth was confirmed by plating on EMB agar. After 48 hours of treatment with oral streptomycin the high streptomycin concentration tubes were found to contain heavy growth. The zone of inhibition, between 156 units/cc. and 2500 units/cc., was also confirmed since plating on EMB agar produced no growth or scant growth.

These observations indicate that resistant strains of *E. Coli* develop with great rapidity within 48 hours after institution of streptomycin.

The fact that *E. Coli* may be inhibited by concentrations of streptomycin below 2500 units/cc. and yet not be killed by concentrations between 2500 units/cc. and 10,000 units/cc. is a phenomenon of considerable interest, and calls attention to a related phenomenon described by Eagle,[11] who observed a similar zone effect with penicillin.

STOOL ASSAY FOR STREPTOMYCIN CONTENT

The stools of three patients who were given 4 Gm. of streptomycin daily divided 1 Gm. every six hours were assayed for streptomycin content. It was found that within 48 hours after the initiation of therapy a level of 4,800 to 9,600 units of streptomycin per gram of wet stool was reached. One patient was found to have 19,200 units/gram after six days of oral streptomycin. It will be noted that these concentrations are greater than the prozone levels observed in the titrations, and are in the range where streptomycin resistant organisms proliferate.

ORAL STREPTOMYCIN COMBINED WITH SULFATHALADINE

Because of the unreliable results obtained with oral streptomycin alone, a series of preoperative patients were given oral streptomycin in combination with sulfathaladine, and daily colony counts of the stools by the method previously described were carried out. Dosages given were 0.25 Gm. of streptomycin and 1 Gm. sulfathaladine every six hours by mouth. Though this series is too small to permit valid conclusions the data on the nine cases so treated are here reported, since they appear to show results essentially similar to the response obtained with oral streptomycin alone (See Table III). It will be noted that only four of the nine cases showed a significant drop in the numbers of coliform and streptococcus colonies.

In all four of these cases the effect was short-lived, the maximum reduction in the bacterial flora occurring within 48 to 72 hours after the institution

of treatment, and thereafter returning very rapidly to the pretreatment level, this level becoming re-established within five days after the institution of chemotherapy and within two days after the maximum drop. Only one of the nine cases showed a significant drop in the number of clostridia colonies, and this was temporary. All nine patients were followed at least four days, the longest being nine days. No toxic effects were observed in this series of cases.

TABLE III.—*Response of Colony Counts to 1.0 Gm. Streptomycin and 4.0 Gm. Sulfathaladine Per Day*

Degree of Response	Number Giving No Response	Number Giving Temporary Response		Number Giving Prolonged Response		Total Number of Cases
		To 0.01%	To 0.001% or Less	To 0.01%	To 0.001% or Less	
E. Coli	5	1	3	0	0	9
Streptococci	5	4	0	0	0	9
Cl. welchii	8	1	0	0	0	9

CONCLUSIONS

A comparison of the results reported in this paper with previous publications has led us to the conclusion that oral streptomycin cannot be recommended for the preoperative preparation of patients requiring large bowel surgery, for the following reasons:

(1) Reduction in intestinal flora is unpredictable and unreliable.
(2) In a significant proportion of the cases which show a favorable early response to oral streptomycin, the organisms rapidly develop resistance to the drug.

Although our series of cases is too small to permit final conclusions to be drawn it would seem that the probable development of resistance to streptomycin by the intestinal organisms would be likely to render the parenteral use of this drug ineffective should the patient develop postoperative complications which might ordinarily be treated with this drug. In view of these observations we feel that it would be wiser to reserve the use of streptomycin for the treatment of complications should they develop rather than to expend the drug effect in the preoperative preparation of the patient.

SUMMARY

1. A review of publications on the use of oral streptomycin in preoperative preparation of surgical cases is presented. The conflicting nature of those reports is emphasized.

2. A series of 24 cases treated at the Presbyterian Hospital with oral streptomycin is presented, together with the methods used to obtain serial quantita-

tion colony counts on the three groups of organisms studied, the coliform group, the streptococcus, and the clostridia.

3. Results obtained were inconsistent. Approximately half of the cases treated showed no response in the coliform group to oral streptomycin, and of the cases showing a response, 50 per cent or ¼ of the total number of cases, showed a prolonged significant response.

4. Eighty-seven and seven-tenths per cent of the cases showed no significant reduction in the number of streptococci present in the stool.

5. Thirty-eight and nine-tenths per cent of the cases showed no significant reduction in the number of clostridia present in the stool.

6. Sensitivity titrations on the *E. Coli* group showed rapid development of resistance to streptomycin, and brought out the fact that many strains of *E. Coli* become able to proliferate in the concentrations of streptomycin which exist in feces.

7. A series of nine cases treated with combined streptomycin and sulfathaladine is presented. The results in this group are also inconsistent.

8. The authors conclude that oral streptomycin is unpredictable and unreliable, and its use in the preoperative preparation of surgical cases is not to be recommended.

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