STERILIZATION OF THE INTESTINAL TRACT BY ANTIBIOTICS AND SUPPLEMENTAL AGENTS*

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A CONSIDERABLE CHANGE has come to pass in surgery of the colon during the last decade. Although primary resection and primary anastomosis theoretically have always been procedures of choice, a prohibitive mortality previously limited their usefulness. That situation has now been almost completely reversed, and the mortality of the one-stage operation has been so greatly reduced that the patient's best interests are served by its performance whenever it is not contraindicated.

Chemotherapy and antibiotic therapy have played an important, though by no means the only, part in this happy development. The correction of dehydration, the maintenance of a proper fluid balance, the correction of anemia by massive blood transfusions, the correction of hypoproteinemia, and numerous advances in anesthesia must all share in the credit.

The first step in the accomplishment of effective antisepsis in gastrointestinal surgery was the preoperative use of relatively nonabsorbable sulfonamides, beginning with sulfanilylyguanidine in 1940.[1] At the present time succinylsulfathiazole and phthalylsulfathiazole are most often used for this purpose.[2] They are highly effective. When they are employed in optimal dosages, the bulk of the stool is greatly reduced and the characteristic fecal odor is often eliminated because of the enormous reduction of the coliform bacterial content of the feces. To be more exact, by this method within five to seven days the coliform bacteria in the feces can be reduced to less than 1,000 organisms per gram of wet stool.

* Read in the Section on Surgery, General and Abdominal, American Medical Association, Ninety-Eighth Annual Session, Atlantic City, June 8, 1949. Since this paper was submitted for publication, November, 1949, we have had the opportunity to evaluate "Combiotic" tablets, kindly prepared by Charles Pfizer and Co., Inc., which combine streptomycin sulfate 250 mg., bacitracin 5000 units, and polymyxin 20 mg., in preparation of the bowel for intestinal resection. Eight tablets a day, divided in 4 doses, to 20 patients, effected substantially the same results as obtained with the streptomycin-glucuronolactone combination, that is, maximal suppression was obtained in 36 to 72 hours. Prolonged administration of "Combiotic" tablets did not prevent the emergence of streptomycin-resistant bacteria. No other untoward effects were observed.

Intestinal obstructions, the presence of ulcerated lesions in the bowel, intestinal perforations, and intestinal fistulas, in general, interfere with the removal of susceptible bacteria from the intestinal tract, regardless of the antiseptic, or combinations of antiseptics, used.

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The sulfonamide drugs, however, have a number of disadvantages. Because large doses are necessary, toxicity as a result of absorption is always a possibility. The action of sulfanilamide and its derivatives is relatively slow. Finally, certain bacteria resident in the intestinal tract are refractory to them.

At least two factors limit the usefulness of penicillin as an intestinal antiseptic. When it is given orally, it is readily absorbed into the bloodstream from the upper digestive tract, while coliform bacteria, which dominate the fecal flora in the lower portion of the intestinal tract are either moderately or completely refractory to penicillin, or produce enzymes which inhibit or destroy its antibiotic action.

Streptomycin has numerous potential advantages over the sulfonamide drugs in intestinal surgery. It is not readily absorbed from the gastro-intestinal tract. Instead, it remains in the lumen of the bowel in high concentration. It is much more effective *in vitro* than the sulfonamides against coliform fecal flora. It does not irritate the intestinal mucosa. Finally, the results in the various clinics[3, 4] in which streptomycin has been used in preparation for resection of the colon indicate that it promptly effects a decrease in the bacterial flora, the number of *Escherichia coli* in the feces being reduced to less than 1000 colonies per gram of wet stool, within 48 to 60 hours after commencement of medication. With continued therapy, the suppression thus achieved may be maintained for five to eight days.

Like the sulfonamide drugs, however, streptomycin has its disadvantages. Its effect on coliform organisms appears to be only temporary and, regardless of the dosage used, the count can be expected to rise again after about five days. Moreover, the advantages of the rapid action of streptomycin are offset to a considerable extent by the development of drug resistance: After several days of treatment, there is frequently noted the emergence of an overgrowth of bacteria which are streptomycin-resistant. Lockwood[5] regards oral streptomycin as unreliable and does not recommend it for the preoperative preparation of surgical cases.

The effective suppression of fecal bacteria by chemotherapeutic and antibiotic methods was promptly found to result in the establishment of optimal conditions for the healing of operative wounds of the bowel.[6] It was also shown experimentally, and confirmed clinically, that the reduction of intestinal bacterial flora lessened the incidence of postoperative peritonitis, had an inhibitory effect on peritonitis which had already developed, and hastened the healing of ulcerations of the intestinal wall.

The emergence of drug-resistant bacterial strains, however, has remained a major problem in the application of chemotherapy and antibiotic therapy, and the investigation reported in this communication was undertaken with the idea of testing the effectiveness of supplementary agents in the prevention of this development.

MATERIALS AND METHODS

This investigation was carried out by administering to normal subjects and to subjects with disease of the large bowel streptomycin, both alone and

in combination with aluminum pectinate and glucuronolactone, according to a fixed routine. The effects of aureomycin, Chloromycetin, and of polymyxin were studied by the same plan.

Aluminum pectinate is a derivative of pectin, which is a hydrophilic colloid of great absorptive properties. Glucuronolactone, an inner anhydride of glucuronic acid, is a relatively stable crystalline substance, which forms spontaneously on dehydration of the acid. Glucuronic acid is normally conjugated in the liver with certain vitamins and sex hormones. It is also the principal agent by which such drugs as aspirin, morphine, camphor, chloral hydrate and the sulfonamides are detoxified in the liver. It is normally found in the blood and urine in conjugated forms. Both glucuronolactone and aluminum pectinate were supplied in two forms, in gelatin capsules containing 0.5 Gm., and in tablets already mixed with streptomycin. There was no observed difference in the activity of the two forms.

It is not yet clear why bacterial suppression of the feces is enhanced by either aluminum pectinate or glucuronolactone. We have no opinion about aluminum pectinate,[7] but are of the opinion that glucuronolactone may reduce the pH of the stool and possibly in this way impair the survival of bacteria.

No toxic reactions were observed from the ingestion of aluminum pectinate, glucuronolactone, streptomycin, or polymyxin, but aluminum pectinate produced fullness and bloatedness which were sometimes extremely annoying. Clinically, glucuronolactone is preferable to aluminum pectinate because it is less bulky, and acts more rapidly.

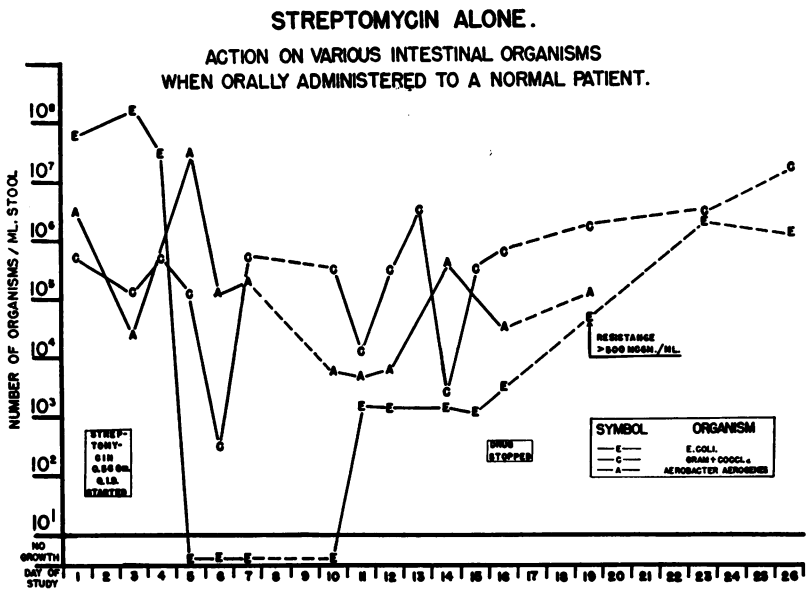
The laboratory procedure was as follows: A known weight of fresh stool, collected in a sterile box, was diluted 50 times in nutrient broth. The suspension was then serially diluted and 0.1 cc. of the diluent was streaked on agar containing blood, eosin methylene blue or sodium azide. Agar plates were incubated aerobically and sodium azide plates anaerobically. The sodium azide plates brought out gram-positive organisms and Bacteroides. Gram-positive organisms identified included hemolytic and nonhemolytic Streptococcus, Micrococcus and Clostridium. The gram-negative organisms included *Escherichia coli,* Proteus, Aerobacter and Pseudomonas. *Esch. coli,* Aerobacter, Streptococcus and Micrococcus were the only organisms present consistently and in sufficient numbers to use in the evaluation of the efficacy of drug action.

In addition to examination of the stools, as described, swabs were taken directly from the mucosa of the colon after resection and the material thus secured was planted on aerobic and anaerobic media. Postoperative bacteriologic studies were continued by the routine employed before operation.

RESULTS

Control Cases. Two healthy subjects were used as controls to furnish a base line for the effect of streptomycin alone on the fecal flora. Each was given 0.5 Gm. orally four times daily for 18 days. Results in both patients were essentially the same (Fig. 1). *Esch. coli* disappeared from the stools

FIG. 1


STREPTOMYCIN ALONE.
ACTION ON VARIOUS INTESTINAL ORGANISMS
WHEN ORALLY ADMINISTERED TO A NORMAL PATIENT.
NUMBER OF ORGANISMS / ML. STOOL
DAY OF STUDY
RESISTANCE >500 MGM./ML.
DRUG STOPPED
SYMBOL ORGANISM
E. COLI.
AEROBACTER AEROGENES

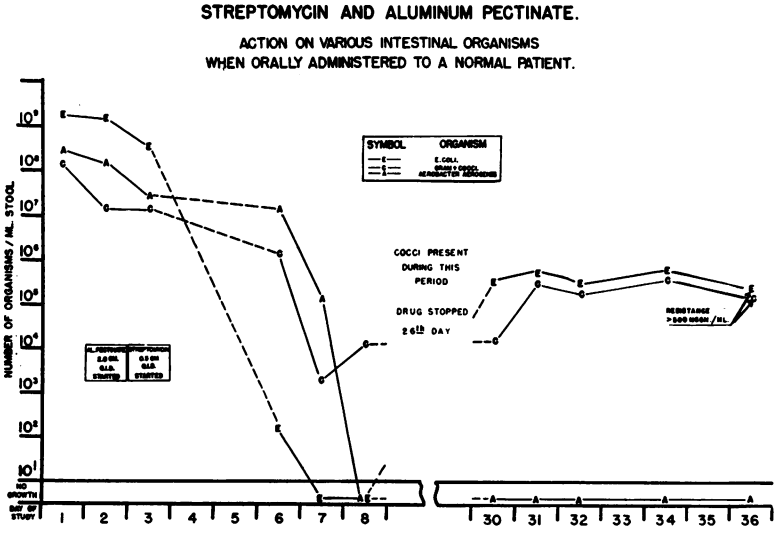

STREPTOMYCIN AND ALUMINUM PECTINATE.
ACTION ON VARIOUS INTESTINAL ORGANISMS
WHEN ORALLY ADMINISTERED TO A NORMAL PATIENT.
NUMBER OF ORGANISMS / ML. STOOL
SYMBOL ORGANISM
COCCI PRESENT DURING THIS PERIOD
DRUG STOPPED
26th DAY
RESISTANCE >500 MGM./ML.
DAY OF STUDY

FIG. 2

within 48 hours. *Aerobacter aerogenes* and gram-positive cocci were not significantly depressed. Drug fast organisms occurred in both subjects six days after maximal suppression, in spite of continued administration of the drug.

Streptomycin-Aluminum Pectinate. Five normal subjects were given 0.5 Gm. of streptomycin combined with 2 Gm. of aluminum pectinate by mouth four times daily for 16 days. Results in all five cases were strikingly similar (Fig. 2). At the conclusion of treatment the stools were normal in color and had the consistency of a semi-gel while the fecal odor was diminished or absent. *Esch. coli* was completely suppressed within 24 to 60 hours after treatment had been begun. *A. aerogenes* (which was not uniformly present) disappeared at about the same time. Most strains of *Esch. coli* were streptomycin sensitive. Except for hemolytic streptococci, gram-positive cocci were not significantly reduced. Clostridium, Bacteroides and yeasts were also not materially affected. Neither *Esch. coli* nor *A. aerogenes* reccurred while therapy was continued, but the organisms which had been suppressed re-appeared, on the average, within 48 hours following the withdrawal of streptomycin.

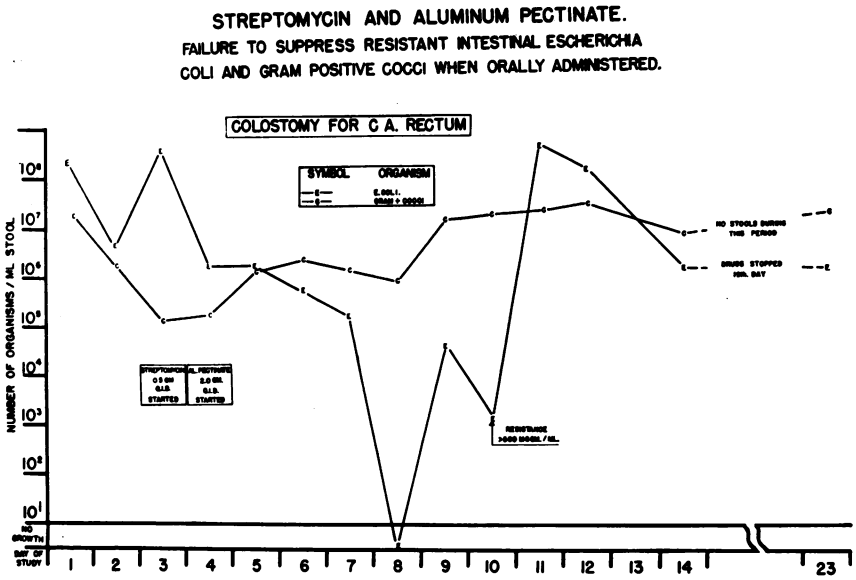
Following this demonstration in normal subjects, six patients with various diseases of the colon were treated with streptomycin and aluminum pectinate by the routine described for four days before resection of the bowel. The last dose was given in each instance two hours before operation, through a Miller-Abbott tube which had been inserted the previous day. Other preoperative preparation consisted of a low residue diet, a dose of castor oil (2 ounces) two days before operation, and repeated enemas over the same period of time.

In four of the six patients, the response was satisfactory. *Esch. coli* and other gram-negative bacilli were suppressed within 60 hours, and there was also a significant reduction in the numbers of gram-positive cocci, particularly the hemolytic streptococci. Clostridium, Bacteroides and Monilia were unaffected. The operative procedure was greatly simplified because the bowel was soft, flat, and readily handled. How soon after operation organisms began to reappear in the feces is not known, since stools were not usually passed before the fourth postoperative day. A liquid specimen removed through the Miller-Abbott tube from the descending colon of one patient on the second postoperative day revealed streptomycin-sensitive *Esch. coli* in small numbers. In the other three cases in this group, the first stools showed a moderate growth of *Esch. coli,* most of which were streptomycin-sensitive.

The fifth patient had an annular carcinoma of the left colon which had caused serious obstruction and the sixth, who had a functioning colostomy, had multiple constrictions of the bowel caused by carcinomatosis (Fig. 3). In both instances, most of the *Esch. coli* found in the intestine were drug-resistant and the response to treatment was therefore poor.

The patient with the colostomy was not operated upon. The other five underwent resection of the colon, with open anastomosis. None developed

FIG. 3


STREPTOMYCIN AND ALUMINUM PECTINATE.
FAILURE TO SUPPRESS RESISTANT INTESTINAL ESCHERICHIA COLI AND GRAM POSITIVE COCCI WHEN ORALLY ADMINISTERED.
COLOSTOMY FOR C A. RECTUM
SYMBOL ORGANISM
NUMBER OF ORGANISMS / ML. STOOL
NO GROWTH
DAY OF STUDY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 23

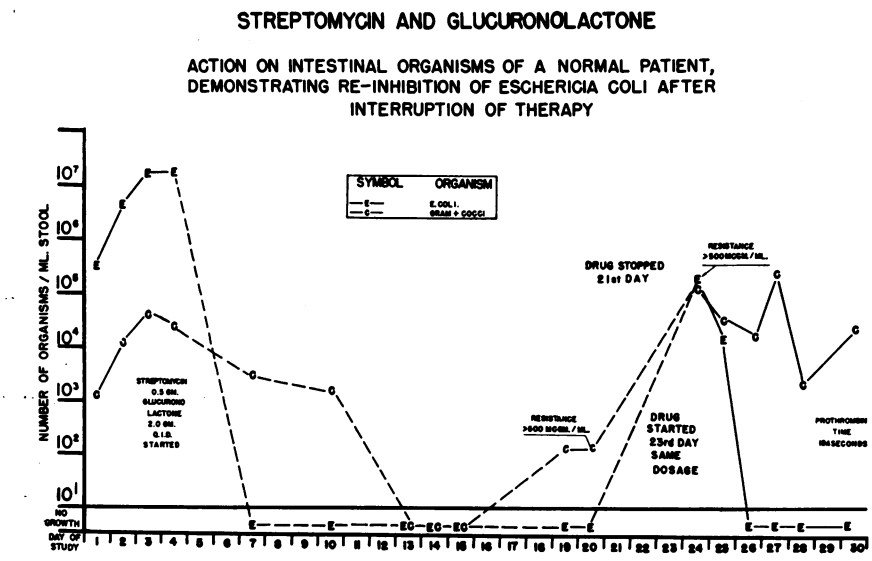

STREPTOMYCIN AND GLUCURONOLACTONE
ACTION ON INTESTINAL ORGANISMS OF A NORMAL PATIENT, DEMONSTRATING RE-INHIBITION OF ESCHERICIA COLI AFTER INTERRUPTION OF THERAPY
SYMBOL ORGANISM
E. COLI.
GRAM + COCCI
NUMBER OF ORGANISMS / ML. STOOL
NO GROWTH
DAY OF STUDY
STREPTOMYCIN 0.5 GM. GLUCURONO LACTONE 2.0 GM. O.I.D. STARTED
DRUG STOPPED 21st DAY
RESISTANCE >500 MCGM. / ML.
DRUG STARTED 23rd DAY SAME DOSAGE
PROTHROMBIN TIME

FIG. 4

peritonitis. Whether this fortunate outcome could be attributed to the preoperative therapy it is not possible to say, since all were given 100,000 units of penicillin by the parenteral route every eight hours after operation, for periods varying from seven to 14 days.

Streptomycin-Glucuronolactone. Eight normal subjects were given 0.5 Gm. streptomycin, combined with 2 Gm. of glucuronolactone, by mouth four times daily for 16 days in seven cases, and for 33 days in one case. In seven of the eight cases coliform bacteria were suppressed within 24 to 48 hours after the beginning of treatment (Fig. 4). In the eighth case *Esch. coli* was not eliminated until the seventh day. In all eight cases suppression was maintained for at least 48 hours after the drugs had been withdrawn. When re-growth occurred, *E. coli* were found to be streptomycin-fast. As to the other organisms, micrococci were reduced to some degree in all subjects and were completely, though temporarily, eliminated in four. Clostridium, Bacteroides and Monilia were unaffected, as were unidentified anaerobic gram-negative rods which were occasionally isolated. It may be that the same results could have been accomplished with a smaller dosage than 8 Gm. daily, but we have not yet carried out tests to determine this point.

Esch. coli re-appeared within 48 hours after treatment had been discontinued in the single case in which glucuronolactone and streptomycin were continued for 33 days. It grew in media containing 0.5 mg. of streptomycin per milliliter. When therapy was reinstituted, the organisms were again suppressed, the inhibition being maintained for another seven days. This course of events suggests that resistant organisms do not permanently dominate the fecal flora following the oral administration of streptomycin.

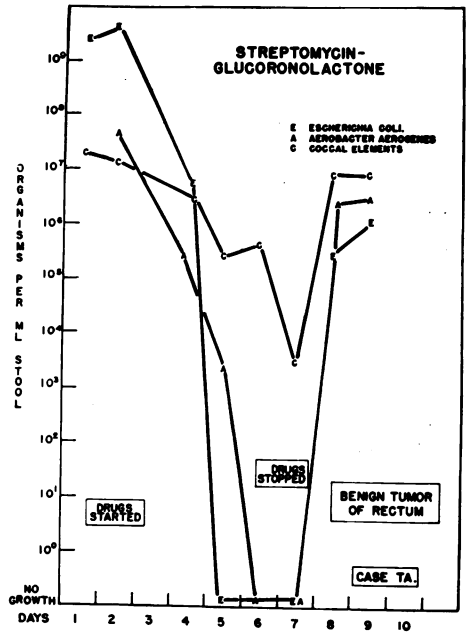


FIG. 5

After this trial in normal subjects, six patients with various lesions of the colon were treated with streptomycin and glucuronolactone by the routine described, for five days prior to resection of the bowel. In all six cases the response was satisfactory (Fig. 5). Susceptible coliform bacteria were suppressed in all instances, maximal suppression being attained in 60 hours in four patients, and by the fifth day of treatment in the other two. Cocci were inconstantly but significantly reduced in numbers and inactive Clostridia and Monilia

appeared unaffected. The time for achievement of maximal suppression, it should be noted, was longer than in the normal subjects.

Aureomycin and Chloromycetin. Aureomycin and Chloromycetin, which became available for study during the course of this investigation, were given to three and to two patients, respectively, in 0.5 Gm. dosages four times daily for 12 days. Gram-positive fecal bacteria were not affected, nor were gram-negative bacteria other than *E. coli.* The suppression accomplished was slight in comparison with that obtained with either sulfonamide drugs or streptomycin. Aureomycin, and Chloromycetin, at least in the dosages used in this study, therefore do not seem to offer much promise as intestinal antiseptics. Both antibiotics are rapidly absorbed into the blood when they are administered orally, but both apparently reach the lower digestive tract in bacteriostatic concentrations for some organisms.

Polymyxin B. This is derived from cultures of Bacillus polymyxa, and became available for testing in the course of this investigation. It is not absorbed from the gastro-intestinal tract, it exerts a rapid bacteriostatic action on many coliform bacteria as well as on certain strains of cocci, and on a weight and dosage basis it is many times more active against coliform organisms than is streptomycin. Proteus strains, in our experience, are indifferent to its action. Drug-fastness does not occur readily.

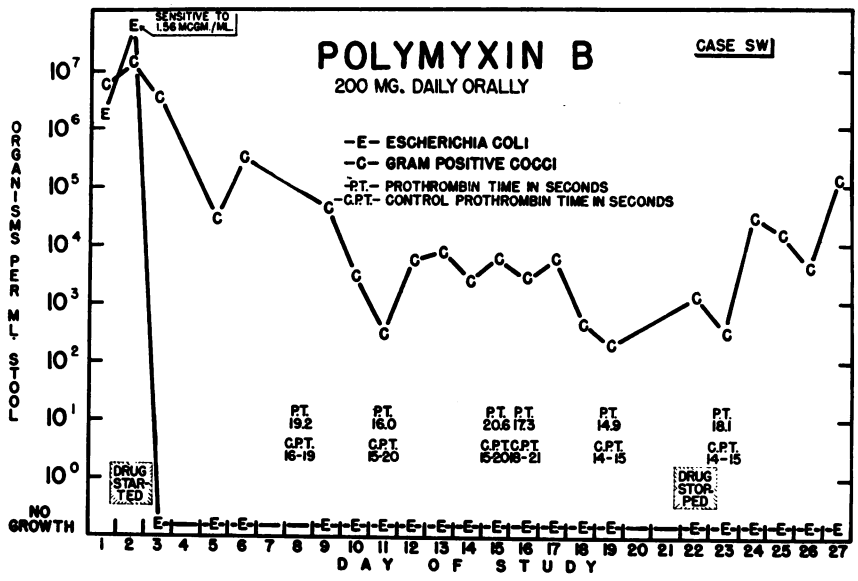
Polymyxin was given orally in divided doses four times daily to five patients who received 200 mg. a day (Fig. 6), and to seven patients who received 400 mg. per day. Treatment was continued for 16 days in the six subjects. Polymyxin was also given for five days to six patients with intestinal lesions (Fig. 7), as part of the preoperative regimen. All coliform organisms were suppressed within 24 to 60 hours after treatment had been begun and suppression was maintained for two days after it was discontinued. The minimally effective dosage appears to be 200 mg. daily. Proteus was present in four of the 12 patients, and eventually dominated the fecal flora. In two of these patients, no additional effects could be attributed to the concomitant administration of glucuronolactone with polymyxin. In each of two others, Proteus could not be recovered after two days of oral streptomycin therapy, 2 Gm. daily.

SUMMARY AND CONCLUSIONS

Tests in 24 normal subjects and in 20 patients with lesions of the colon demonstrate that streptomycin, in dosages of 0.5 Gm. four times daily, eliminates coliform bacteria from the feces at the end of two to four days' treatment. It cannot suppress fecal cocci, clostridia and yeasts and it inhibits Aerobacter only inconstantly. Because of emergence of drug-fast bacteria, maximal suppression can be maintained by streptomycin alone for not more than four to six days.

When glucuronolactone is given in combination with streptomycin, the period of suppression is lengthened to beyond 14 days or more. Aluminum

FIG. 6


POLYMYXIN B
200 MG. DAILY ORALLY
CASE SW
SENSITIVE TO 1.56 MCGM./ML.
-E- ESCHERICHIA COLI
-C- GRAM POSITIVE COCCI
-P.T.- PROTHROMBIN TIME IN SECONDS
-C.P.T.- CONTROL PROTHROMBIN TIME IN SECONDS
ORGANISMS PER ML. STOOL
NO GROWTH
DRUG STARTED
DRUG STOPPED
P.T. 19.2 C.P.T. 16-19
P.T. 16.0 C.P.T. 15-20
P.T. P.T. 20.6 17.3 C.P.T. C.P.T. 15-20 18-21
P.T. 14.9 C.P.T. 14-15
P.T. 18.1 C.P.T. 14-15
DAY OF STUDY

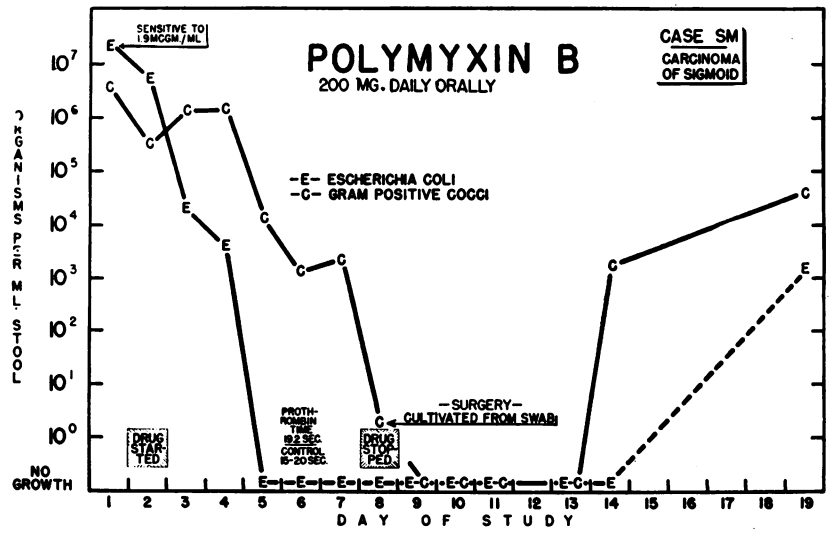

POLYMYXIN B
200 MG. DAILY ORALLY
CASE SM
CARCINOMA OF SIGMOID
SENSITIVE TO 1.9 MCGM./ML.
-E- ESCHERICHIA COLI
-C- GRAM POSITIVE COCCI
ORGANISMS PER ML. STOOL
NO GROWTH
DRUG STARTED
PROTHROMBIN TIME 19.2 SEC. CONTROL 15-20 SEC.
-SURGERY- CULTIVATED FROM SWAB
DRUG STOPPED
DAY OF STUDY

FIG. 7

pectinate with streptomycin gives less consistent responses than the glucuronolactone-streptomycin combination.

Polymyxin B, in total daily dosages of 200 to 400 mg., eliminates all coliform bacteria except Proteus from the feces as rapidly as streptomycin. Aureomycin and chloromycetin in daily doses of 2 Gm. a day do not appear promising as agents for the preoperative preparation of surgical cases.

Regardless of the type of chemotherapeutic treatment used, maximal suppression of intestinal bacteria occurs 24 to 48 hours later in patients with lesions of the colon than in normal subjects.

The successes of antiseptic measures in the suppression of the bacterial flora of the large intestine must not blind the surgeon to the fact that this form of therapy is only one of several measures responsible for the recently reported reduction in the mortality and morbidity of surgery of this part of the intestinal tract. Replacement therapy, including massive blood transfusion, and intestinal decompression have played at least as important a part in this fortunate outcome.

The aluminum pectinate and glucuronolactone used in this investigation were kindly supplied by the Commercial Solvents Corporation, and the polymyxin by Burroughs Wellcome and Company.

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NOTE: See letter to editor on page 319 for a discussion of this article.