A Quantitative Study of the Alterations in the Fecal Flora of Man Following Oral Administration of Aureomycin

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A QUANTITATIVE STUDY OF THE ALTERATIONS IN THE FECAL FLORA OF MAN FOLLOWING ORAL ADMINISTRATION OF AUREOMYCIN

by

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W.P.L.
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INTRODUCTION

The purpose of this investigation was to make a quantitative study of the alterations in the fecal flora of normal healthy subjects following oral administration of aureomycin. Fecal specimens from these subjects were studied daily before medication, during medication and following the medication for a varying number of days until the fecal flora had returned to pre-drug levels. The data are herewith presented, analyzed and evaluated.
REVIEW OF THE LITERATURE

Since the discoveries of various antimicrobial agents (chemotherapeutic agents) their effects on the intestinal flora of man and lower animals have become increasingly interesting to microbiologists, gastroenterologists, abdominal surgeons, agricultural researchers, and nutritionists. This review of the literature is intended to cover briefly all the important work thus far done on the alteration in normal intestinal flora of man and animals treated with the commonly used antimicrobial agents. Emphasis will be given with reference to aureomycin and terramycin. The problem of developing bacterial resistance toward these agents will be mentioned wherever indicated.

Tyrothricin

As early as 1941, Weinstein and Rammelkamp (132) reported that tyrothricin had no effect on Lactobacillus acidophilus in vivo even when 160 times the in vitro killing dose was given to mice. Two possibilities were formulated to explain this discrepancy. One is that tyrothricin may be destroyed by the pancreatic or intestinal juices of the animal. Another possible explanation for the failure of tyrothricin to kill L. acidophilus in the living animals may be that the bactericidal agent is inhibited by the fecal material. Tyrothricin was not found to produce any local lesions in the gastrointestinal tract of the animal in this study. No similar study with tyrothricin has appeared in the literature since.

Sulfonamides

The effect of sulfonamides on normal intestinal flora did not become a subject of extensive study until the poorly absorbed sulfonamides were discovered. Most of the studies done in this regard are either as a part
of nutrition experiments on animals or in an attempt to find a suitable antimicrobial agent for the intestinal antisepsis in man. The latter has been of particular interest to surgeons looking for possible measures to cut down the incidence of septic complications following operations on the gastrointestinal tract. Much effort has been expended in the hope of finding a poorly absorbed sulfonamide which would alter significantly the bacterial flora of the gastrointestinal tract without adversely affecting the host. Marshall and his associates (74) were probably the first group of investigators to report, in 1940, that marked reduction of coliforms (in man and mice) followed oral administration of sulfanilylguanidine. Firor and Poth (41) later indicated that this compound did not decrease the number of bacteria other than coliforms in the colon of man and the dog and there was no reduction even in coliforms in presence of ulcerative lesions of the bowel. The ineffectiveness of sulfaguanidine in presence of ulcerative colitis was later confirmed by Vieta and Stevenson (128).

In 1941 Poth and his associates (92, 93, 94) found that succinylsulfathiazole (sulfasuxidine) markedly diminished the coliforms in the bowel of man and the dog in 1 to 7 days in 95 per cent of cases. This marked reduction in the number of coliforms was supported by Welch and his associates (135), Gant and his associates (43), Lockwood (70) and others (31, 6). Lockwood (70) found that in addition to the marked reduction (from $10^{11}$ to $10^5$) of the gram-positive bacteria in certain clinical cases as previously described by Poth and his co-workers (94) the drop in number of clostridia (welchi) paralleled rather closely the drop of coliforms and that there was an increase in the total number of streptococci during the succinylsulfathiazole treatment. Firor (39), Kirby and Kantz (65), and
Gant and his associates (43) reported a similar increase of enterococci during medication. Gant and his associates (43) also found that in rats given sulfaguanidine or sulfasuxidine the coliform counts remained low for 10 days and then began to increase and at the end of the five weeks of medication almost approached the normal pre-drug level. This increase of coliforms or *Escherichia coli* during the later period of medication was later confirmed by Evenson and his co-workers (31) who also reported increase of yeast-like organisms during medication. Archer and Lehman (6) showed that the presence of ulcerative lesions did not interfere with the effect of sulfasuxidine and that the patients receiving a considerable amount of protein in their diet presented a course of more rapid decline of coliforms than those without.

Fior and Jones (40) first used sulfanilylguanidine in the pre-operative preparation of 12 patients subjected to surgical procedures on the colon and reported gratifying results. Allen (1) remarked upon the unusually smooth convalescence following combined abdominoperineal resection after the use of sulfasuxidine. Poth (86) in a review of therapeutic agents for intestinal antisepsis re-emphasized the value of sulfasuxidine as a useful adjuvant in surgery of the colon and formulated an outline for the proper use of this drug. A few more poorly absorbed sulfonamides were then discovered from time to time. Callomon and Raiziss (16) reported in 1943 that the bactericidal effect of succinyl sulfapyrazine is much greater than that of sulfasuxidine and is at least equal to or greater than that of sulfaguanidine. Poth and Ross (97, 98 85) found that phthalylsulfathiazole (sulfathalidine) had 2 to 4 times the bacteriostatic activity of sulfasuxidine in the bowel of man or dog.
Microorganisms were usually greatly decreased in 24 hours and coliforms were ordinarily reduced to less than 1,000 per gram of wet feces within 3 days. Further studies of Poth and Ross (99, 87) showed that sulfathalidine caused more rapid reduction of coliforms and clostridia, less tendency to form semi-liquid stools and were better suited to patients with ulcerative colitis and watery diarrhea than sulfasuxidine. The enterococci were however not affected. Among a few reports of recent years, Dearing and Heilman (20) claimed that sulfasuxidine and sulfathalidine are far from being efficient as antibacterial agents against E. coli. Furthermore, Streptococcus fecalis always tended to increase in number as the medication was continued and clostridia were present in the culture eventually in 21 of 35 patients during adequate medication.

Ganey and his associates (42) in 1950 presented evidence that during sulfasuxidine therapy (0.25 gram per kilo per day for 7 days) the reduced coliforms started to increase in number on the 4th day of the treatment and reached a level much higher than the initial pre-drug level on 5th day after discontinuance of the drug. There was considerable increase of Proteus and clostridia in addition to the increase of streptococci previously reported by many other investigators. The same group of workers also found formosulfathiazole to be a much better bacteriostatic agent on intestinal flora. Formosulfathiazole is a condensation product of sulfathiazole and formaldehyde. It is poorly absorbed from the intestinal tract. Seven out of 10 patients given 2 grams of this drug orally every 8 hours for 7 days showed a marked reduction in coliforms and total organisms at the end of 72 hours of medication. This drug also reduced the number of Proteus and streptococci in addition to the clostridia.
This inhibitory action lasted over a period longer than 5 days after discontinuance of the drug. The total bacterial counts, however, started to go up slowly after the 4th day of medication. Zenker and Groll (140) advised the use of the combination of formosulfathiazole and streptomycin to give a better bacteriostatic effect. The suppressive effect of formosulfathiazole upon Proteus was recently confirmed by the work of Marmell and his co-workers (73) who also found a decrease in aerobic and anaerobic spore-forming bacteria and no increase in yeast-like organisms. Following a temporary decrease in number, the enterococci, however increased tremendously during medication with formothiazole and might replace all the other flora.

The development of resistance to sulfonamides by the intestinal flora has not been as serious a problem as the development of resistance to streptomycin. Kirby and Rantz (64) showed by in vitro studies that the resistance of \textit{E. coli} to sulfonamides developed gradually and could be demonstrated for all 4 drugs tested: sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine. A common structural unit possessed by these drugs and an interaction involving the same enzyme system were suggested to explain the development of cross resistance. Harris and Kohn (50) pointed out that the resistance of the bacterium not only depends upon the medium in which "training" occurred, but also upon the medium in which the measure of resistance is made.

\textbf{Penicillin}

Few studies concerning the effect of penicillin on the intestinal flora have been made, probably because of the insusceptibility of the predominating gram-negative bacteria of bowel to this antibiotic.
Thomas and Levine (125) in 1945, found that *in vitro* the gram-negative bacilli of the intestinal tract seemed to fall into the following order of descending susceptibility to penicillin: *Salmonella*, *Proteus*, *Shigella*, *Escherichia*, and *Aerobacter*. These organisms were affected only when the concentration of penicillin was extraordinarily high. They also reported changes in cultural characters and the development of bizarre involution forms. These varied from large, long, twisting filaments to cells resembling deformed integral signs, Pasteur flasks, swelled fusiform bacilli, large globular cells and irregular masses. Weinstein (131) in 1946 succeeded in producing complete suppression of the growth of cultures containing standardized inocula of both *E. coli* and *Staphylococcus aureus* with 3 per cent urethane or 6 per cent urea combined with 0.1 unit of penicillin. Lipman and his associates (67) reported in 1948 that penicillin given to arthritic patients caused a lower incidence of enterococci but showed no noticeable effect on the enteric bacilli and the anaerobes. Anderson and his co-workers (3) reported in 1952 that in a study of chicks fed a diet containing 10 p.p.m. of penicillin and 23 per cent of protein, the antibiotic caused a reduction in pH of the cecal contents, increase of lactobacilli, aciduric organisms and coliforms, especially atypical *E. coli*, and some reduction of enterococci. The drug also markedly increased the size of ceca and enhanced the oxygen intake of lactobacillus and aciduric types of organisms. Recently Guzman-Garcia and his associates (47) found that in rats fed penicillin (25 mg per kilogram) there was a marked increase in the number of coliforms (about 100 times) in the cecum and small intestine from the 7th day of the treatment through the 28th day. The numbers of anaerobes declined during the first
week but the effect disappeared after 2 weeks. There appeared to be slight reduction of lactobacilli. Enterococci were not altered. In the presence of a diet deficient in thiamine, the drug caused a marked increase in the growth of the rats.

**Streptomycin, Neomycin, Polymyxin and Bacitracin**

Before the rapid development of bacterial resistance against streptomycin became a serious problem, streptomycin appeared very promising for suppressing the predominately gram-negative intestinal flora. Smith and Robinson (18) reported in 1945 that streptomycin was much more effective than streptothricin, sulfaguanidine, and sulfasuxidine in both the rate and the extent of reduction of the number of intestinal organisms in mice. Within 24 hours after the beginning of treatment with 30,000 units of oral streptomycin per kg per day the coliform count decreased 1,000-fold and the numbers of non-lactose fermenting organisms decreased 200-fold. When the streptomycin dosage was raised to 300,000 units per kg per day, it eliminated not only all coliforms, but all gram-negative organisms, leaving only a small number of gram-positive spore-formers such as Bacillus subtilis. This decrease or elimination of organisms persisted throughout the 3-week period of treatment. Emergence of Pseudomonas aeruginosa and staphylococci was not encountered. Kane and Foley (62) in 1947 found that oral administration of as little as 1.0 g of streptomycin daily eliminated E. coli from the stools of 5 patients within 2 days. The fecal streptococci, clostridia, Bacteroides, and Candida were unaffected. They concluded that streptomycin is much more effective than sulfonamides in ridding the colon of E. coli for preoperative preparation of patients who are to undergo surgery of the colon. This view was supported by Zintel
and others (142) who in addition claimed that this antibiotic also decreased the number of Str. faecalis and clostridia. Rowe, Spaulding and their co-workers (108, 120) reported that the combination of sulfathalidine and streptomycin caused more rapid reduction of the coliform count than either drug used alone. This was, however, not confirmed by Poth and his associates (98) and Lockwood and his colleagues (71). Furthermore, Lockwood and his colleagues advised against the use of streptomycin for preoperative use because of the risk of the development of resistance and the establishment of a highly resistant flora at the time of operation. Spaulding and his associates (120) pointed out that the combination of streptomycin and sulfathalidine did not prevent the development of streptomycin resistance and in 2 cases treated with this mixture there was a definite reversion of the low coliform count due to rapid proliferation of resistant Aerobacter aerogenes while the treatment was still continued. The increase of coliforms began on 5th day of the treatment in one instance and on the 9th day in the other. De Van (19) in a study of 40 patients treated preoperatively with 1 gram of streptomycin by mouth every 6 hours for 5 days found that the stool became sterile for aerobic and anaerobic organisms in 37.5 per cent of cases at the end of 48 hours and in 47.5 per cent after 72 hours. The earliest time for the resistant organisms to appear was 4 to 5 days after the beginning of the treatment. This time limit agrees fairly well with the findings of Spaulding and his associates (120) and Pulaski and his associates (104). The preoperative use of streptomycin was therefore advised to last not longer than 2 or 3 days and moderates doses of Vitamin K should be given to counteract any tendency to develop hypoprothrombinemia.
Pulaski and his associates (104) reported in 1950 that when 8 normal subjects and 6 patients were given 0.5 g streptomycin, combined with 2 g of glucuronolactone, by mouth 4 times daily, E. coli was completely suppressed within 24 to 48 hours and the period of suppression could be lengthened to 14 days or more. Micrococci were reduced in number inconstantly but significantly. Clostridia, Bacteroides and Candida were unaffected. E. coli reappeared within 48 hours after the treatment had been discontinued in a case treated for 33 days but was again suppressed for another 7 days when the therapy was reinstituted. The advantage of combining streptomycin with glucuronolactone was confirmed by Welch and his co-workers (136). On the other hand, Donaldson and Bricker (28) reported that only 40 per cent of patients showed reduced numbers of coliforms and 65 per cent showed reduced numbers of streptococci and that in 70 per cent of patients either the coliform or the enteric streptococcal bacteria developed streptomycin-resistant strains within the first 48 to 96 hours after the start of this combined therapy, as evidenced by the return of their number to normal or higher than normal.

Neomycin isolated from Streptomyces fradiae was first described by Waksman and Lechevalier (130) in 1949 and soon proved to be much more promising than streptomycin for alteration of intestinal flora. Poth and his associates (88, 90, 91, 95) found that in man and dog treated with 0.1 gram per kilo of body weight per day all the culturable bacteria could be eliminated from the gastro-intestinal tract within 24 hours. This new antibiotic did not favor the development of resistant strains but in approximately 10 per cent of patients it failed to inhibit the growth of
A. aerogenes. Therefore, combined therapy with sulfathalidine was suggested for preoperative use to inhibit A. aerogenes. Either this combined therapy or neomycin alone still failed to inhibit the growth of the yeasts.

Finegold (32) claimed that oral administration of bacitracin could profoundly affect Str. faecalis and clostridia and that apparently low counts could be maintained for at least 2 or 3 weeks on continued treatment. He also found that the combination of streptomycin and bacitracin offered the better results than those obtained with either antibiotic alone. Welch and his co-workers (136) reported promising results with a combination of bacitracin and streptomycin in obtaining a greater reduction of coliforms and with Polymyxin B to counteract the streptomycin-resistant organisms. This streptomycin-bacitracin-polymyxin mixture could completely eliminate the coliforms by the 4th day of the medication and the coliforms remained absent until 3 days after discontinuance of the medication. Poth (88) recently concluded that bacitracin is of little value, when used alone. It is mainly effective against gram-positive bacteria including penicillin-resistant varieties (10, 104).

The poorly absorbed Polymyxin B is extremely active against many gram-negative bacilli especially Pseudomonas aeruginosa and streptomycin-resistant coliforms. Pulaski and his associates (103, 104) reported that Polymyxin B, in a total daily oral dosage of only 200 to 400 mg, could eliminate all the coliforms as rapidly as streptomycin but the cocci, Proteus and clostridia appeared to be unaffected. Drug-fastness did not occur readily. Recently Jawetz and Bierman (58) found that in patients treated with a daily oral dosage of 400 mg of Polymyxin B, within 24 to 72
hours there was a reduction of the coliform count to 1,000 or less per gram of wet feces, together with some reduction of total anaerobes and an inconsistent suppression of the spore-forming anaerobic bacilli. This suppression lasted for 2 to 5 days after the oral medication had been discontinued. Enterococci were not significantly affected. A mixture of polymyxin with either neomycin or bacitracin appeared to be much more active than the single components. They markedly suppressed the enterococci, Proteus and Pseudomonas in addition to complete suppression of the coliforms. Polymyxin-resistant coliform was encountered during medication in one instance in their series of 14 patients.

Since the development of drug-resistant organisms has become a serious problem, especially in the case of streptomycin, a tremendous amount of research has been done in an endeavor to learn the nature of the development of resistance. Since this investigation is not concerned with this problem, it will not be discussed here. For information concerning antibiotic resistance see the papers published by Braun(11), Demerec (25, 26), Sevag and Rosanoff (116), Gibson and Gibson (46).

In addition to the development of drug resistance, the spontaneous occurrence of new infections due to insusceptible organisms originally present in the patient's body is by no means rare. Weinstein (133) in 1947 described 5 such cases. In one patient who probably had atypical virus pneumonia, the administration of penicillin resulted in an overgrowth of Hemophilus influenzae in the pharynx, followed by an invasion of the blood and respiratory tract. In another penicillin-treated patient there was spontaneous occurrence of pneumonia due to Friedländer's bacillus. The remaining 3 cases were treated with streptomycin for H.
influenzae infections, and one developed bronchopneumonia with bacteremia, another meningitis with bacteremia, and a third recurrent pyelonephritis, all due to hemolytic Staph. aureus. These superinfections are merely a result of rapid proliferation and invasion of the normal inhabitants following reduction or elimination of the real infectious agents. More illustrations will be given when the other antibiotics are reviewed.

**Chloromycetin, Aureomycin and Terramycin**

The advent of these broad-spectrum antibiotics has made the hope greater than ever before that the administration of a single antimicrobial agent could eliminate from the intestinal flora as many species as possible.

Chloromycetin (Chloramphenicol) is not generally considered to be a good preoperative intestinal antiseptic (68, 106). Ganey and his associates (42) noted an increase in Proteus and clostridia during chloromycetin treatment. Bierman and Jawetz (9) found an increase of staphylococci, Pseudomonas, and yeasts during the therapy. Pulaski and his associates (101, 102, 103) did not consider chloromycetin promising, as it suppressed only E. coli among the normal intestinal flora. They also noted the return of the E. coli and eventual increase of the total bacterial count to above pre-drug levels. Dearing and Heilman (20) gave chloromycetin in doses of 750 mg 4 times daily for from 2½ to 12 days, but did not find it reliable or effective.

Aureomycin (chlortetracycline) and terramycin (oxytetracycline) are very similar in structure as well as in their spectra of antibiotic activity. Conflicting reports have appeared concerning the ability of these antibiotics to reduce the bacterial population of the intestine. Many investigators have published favorable findings. Early reports by
Finland and his associates (36, 37) indicated that as a prophylactic agent aureomycin had proved superior to the combination of streptomycin and the poorly absorbed sulfonamides. The total bacterial counts of both Gram-positive and Gram-negative organisms in the lower bowel contents dropped rapidly and remained persistently low for long periods during oral administration of aureomycin. Neter (82), in a few cases treated with aureomycin, was able to sterilize the bowel contents. Dearing and Heilman (20, 21) reported in 1950 that all the culturable bacteria except Pseudomonas and Proteus were removed within a period of from 1½ to 6½ days from the start of oral aureomycin therapy in 75 out of 91 patients. They concluded that aureomycin showed great promise and superiority over many other antimicrobial agents including chloromycetin, dihydrostreptomycin, sulfasuxidine, and sulfathalidine studied at the same time. During the same period Baker and Pulaski (7) and DiCaprio and Rantz (27) published favorable reports on the preoperative use of terramycin. Baker and Pulaski found that the coliform bacteria were rapidly eliminated in 10 of 11 patients and streptococci were eliminated in 6 of the 11 patients, and suppressed in 2 others. McVay (78) reported in 1952 that in 9 patients treated with one gram of aureomycin daily for 2 days, marked or complete inhibition of 7 out of the 8 kinds of bacteria studied occurred within 24 to 48 hours and the definite reduction of these organisms persisted for 2 days after medication had been discontinued. Using 3 g of the drug daily for 2 days, he was able to demonstrate complete inhibition of 6, including E. coli, anaerobic streptococci, Bacteroides, Str. viridans, Str. pyogenes, and Clostridium perfringens. Str. faecalis and A. aerogenes were not uniformly inhibited. Dearing, Mann and Needham
(23) found that in 14 patients treated with 3 g of terramycin daily for from 3 to 5 days there were similar changes and in addition there was disappearance of yeasts and Proteus in a majority of the cases. Andina and Allemann (5) and Broitman and his associates (13) advocated the use of the less soluble amphoteric terramycin in smaller doses to get a greater antibiotic activity in bowel. Other favorable reports concerning studies upon man with these antibiotics were given by Sborov and his associates (111), Rivera and Sborov (107) and Riddell (106). Studies of poult's, chicks and rats (4, 30, 112, 113, 117, 119) have shown marked alterations in fecal flora similar to those in man. These antibiotics in proper dosage (small) could also lead to rapid and extra growth of pigs, calves, rats, chicks, turkey poult's, etc. (4, 30, 59, 61, 109).

It has been postulated that these antibiotics may promote growth in that they conserve a given vitamin for the host by inhibiting or reducing the intestinal flora which would compete with the host for that vitamin.

In contrast to all the favorable reports mentioned above, many other investigators have found less satisfactory or unsatisfactory results with these two broad-spectrum antibiotics. Metzger and Shapse (76) reported in 1950 that, in their experience, administration of aureomycin did not cause a significant diminution of any prominent member of the intestinal flora. They concluded that administration of aureomycin alone does not appear to be effective in reducing the colonic flora prior to surgery, and furthermore that the suppression of coliform flora may be detrimental by allowing the development of a highly resistant flora. Ganey and his associates (42) found that only one out of ten patients treated with aureomycin, 3 g per day, and 5 out of 10 patients
treated with the same dosage of terramycin showed a reduction in coliforms. These patients at the same time showed an increase in the total colony counts. Less satisfactory results with terramycin and aureomycin were also reported by Wright and Prigot (138) and Dearing and Needham (24) during 1951. Poth (88, 89) in 1952 pointed out that these antibiotics are inferior to neomycin for intestinal antisepsis. Furthermore they cause untoward gastrointestinal reactions and rapid growth of insusceptible yeasts and *Staph. aureus*. Among a few recent reports Morton, Riddell and Murray (81) commented that the actual effect of terramycin alone on the reduction of the fecal flora is of no value. Although there was a reduction in the number and species of coliforms in the 10 patients they studied, the total bacterial counts remained at high levels, even with doses as large as 2 or 3 g a day. In addition, the antibiotic-resistant Proteus and *Pseudomonas* appeared in a great many of the cases and completely dominated the subsequent fecal flora. Tyson and his co-workers (127) in studying 19 patients treated with 1.5 or 3 g of terramycin per day presented a similar picture. The essential change was a replacement of *E. coli* by enterococci, Proteus, and yeasts. There was an initial small drop in total count followed by an elevation of total count to above the pre-drug level. Lactobacillus and *Aerobacter* were eliminated or reduced. The predominating enterococci showed a rapid increase in resistance. They remarked that this alteration of the fecal flora, with an increase in numbers, and consisting of organisms resistant not only to the drug used but to 2 similar broad-spectrum antibiotics is certainly undesirable. More recently Allen’s (2) study of 48 patients treated with 1 to 4 g of terramycin daily has further substan-
tiated the findings of the previous investigators. He found that oral administration of terramycin did not sterilize the colon. The "total" number of bacteria in the intestinal contents was actually increased due to greater numbers of streptococci, yeasts and Proteus during the treatment. Resistant strains of coliforms and lactobacilli appeared during the second week of the therapy.

Among the disadvantages of the use of these broad-spectrum antibiotics as intestinal antiseptics, the rapid proliferation of resistant organisms to cause severe superinfections is far more important than the toxicity and ineffectiveness of the drugs and has become a great concern to us. The rapid proliferation of the following drug-resistant organisms has been commonly encountered as serious complications of the antibiotic therapy:

Pathogenic staphylococci

Finland and his colleagues (35) reported in 1951 that diarrhea associated with recovery of pathogenic staphylococci in large numbers and sometimes in pure culture had been noted in some patients during antibiotic therapy. The same group of investigators (55) again reported that 37 of 91 patients treated with terramycin for pneumonia developed diarrhea; cultures of the stools were made in 18 of these patients and in 12, Staph. aureus resistant to penicillin, terramycin, and aureomycin was found as the only or the predominant organism in the watery feces. The staphylococcal diarrhea may have been an important contributing factor in the fatal outcome of at least 3 of these patients. During the next year Janbon and his associates (56, 57) in France observed 3 deaths out of 9 patients who manifested a choleriform diarrhea associated with staphylococci during
terramycin treatment for various infections. More reports concerning staphylococcal diarrhea have appeared since 1953. Dearing and Heilman (22) presented 44 patients, in 43 of whom staphylococci were found in stool cultures; 39 of these had received terramycin; succinylsulfathiazole, penicillin in addition to dihydrostreptomycin, and aureomycin had each been used in 1 patient, and 2 had no antimicrobial therapy. A majority of the patient having staphylococci developed diarrhea. Death following the staphylococcal diarrhea occurred in 6 cases, 5 of these having received terramycin and 1, aureomycin. A 7th terramycin patient died of severe diarrhea and shock due to pseudomembranous ileocolitis, but no staphylococci were found on cultures. Other cases of severe diarrhea due to drug-resistant staphylococci or suspected to be so following the use of broad-spectrum antibiotics include 8 cases from Terplan and his associates (24), 2 cases from Gardner (44), 7 cases from Reiner and others (105), 2 cases from Bernhart (8), 3 cases from Meier (80) etc. Very recently Finland and his associates (34) reported that among 520 patients treated with terramycin or aureomycin hemolytic Staphylococcus aureus was found as the only or predominant organism in one or more cultures from 27 of the 38 terramycin-treated patients and from 4 of the 22 aureomycin-treated patients; all of them had diarrhea during medication. There were 2 and probably 6 additional terramycin-treated patients in whom staphylococcal diarrhea may have contributed to their fatal outcome. Only one aureomycin-treated patient could fall into this category. The incidence of strains of staphylococci resistant to penicillin and to these broad spectrum antibiotics has been rapidly increasing almost everywhere with the extensive use of these antimicrobial
agents (33, 66, 134). They could be widely spreading as evidenced by the findings of Dowling and others (29). To suppress these drug-resistant strains, Dearing and Heilman (22) and Haight and Finland (48) found erythromycin promising. Recently, Spink (121) advocated combining erythromycin with bacitracin. Howe (53) has noted favorable results with carbomycin in a few clinical trials.

**Yeasts**

Inflammatory lesions of mucous membranes following prolonged use of these antibiotics were at first considered to be avitaminosis caused by suppression of the intestinal flora (83). Probably the yeasts were not incriminated until the time when Farber (141) reported a case of disseminated moniliasis following antibiotic therapy and Harris (49) isolated *Candida albicans* from 5 of 25 patients who had had acute inflammatory disease of mucous membranes while receiving aureomycin and/or chloromycetin. A number of similar cases including a few cases of fatal generalized moniliasis were then reported by Mangiaracine (72), Gausewitz et al. (45), Woods et al. (137), Vorreith (129), Brown and others (14). Pappenfort and Schnall (84) reported in 1951 that aureomycin in vitro could cause a definite stimulation of the growth of *C. albicans*. Later, Seligmann (114, 115) was able to demonstrate that both aureomycin and terramycin could enhance the virulence of *C. albicans* for mice. Huppert and his associates (54) confirmed in vitro the growth stimulating effect of aureomycin, but not terramycin, on *C. albicans*. Recently, McGovern and his colleagues (77) reported that in a group of 45 children given aureomycin or chloromycetin orally for from 8 to 14 days the incidence of *C. albicans* in the gastrointestinal tract increased
from 17 per cent before the treatment to 33 per cent at the end of the treatment period. Loh and Baker (68) have noted that one subject showed a 100-fold increase in yeasts even on as little as 20 mg of aureomycin per day by mouth. The subject, however, did not show any untoward reactions from the overgrowth of the yeasts.

Proteus

The frequent emergence and overgrowth of Proteus following the oral administration of these broad-spectrum antibiotics has been universally observed (2, 7, 12, 17, 42, 57, 76, 81, 107, 117, 138, 139). Carrère and Roux (17) noted in 1951 that 4 guinea pigs given 20 mg of terramycin per day for each animal developed malnutrition and cachexia coinciding with the appearance of Proteus in their feces. Proteus was also isolated from the blood and various internal organs of 2 of the animals after their death. Yow (139) reported in 1952 that 15 cases of urinary infections, 2 cases of bacteremia, and one case each of meningitis and peritonitis, all due to overgrowth of Proteus following oral use of antibiotics, especially the broad-spectrum antibiotics, had been observed during a 2-year period. Loh and Baker (68) have reported a case who developed a dysentery-like diarrhea after receiving aureomycin. Several stool cultures from the subject proved the absence of members of either the Shigella or Salmonella groups. In fact, cultures made on the usual media employed for isolation of enteric pathogens gave pure cultures of Proteus on several occasions. Examination of his stools for parasites revealed nothing. Fortunately, the symptoms gradually subsided within about 3 weeks following withdrawal of the aureomycin. These results led to the conclusion that the diarrhea may have been caused by the overgrowth of
aureomycin-resistant Proteus. It was also because of the stimulation from these findings that the writer has been greatly interested in pursuing the present investigation in the hope of getting a more detailed knowledge of the quantitative changes in the fecal flora following oral administration of aureomycin as well as looking for more evidence for the role of Proteus in causing dysentery-like diarrhea following the medication.

**Pseudomonas**

The overgrowth of the drug-resistant *Pseudomonas* during or following the oral use of these antibiotics has also been noted by many investigators (7, 52, 81, 107). Yow (139) has observed 37 cases of *Pseudomonas* infections, mostly urinary infections during or following the use of antibiotics during a 2-year period of clinical experience. Four of the 8 illustrative cases presented may have some relation with the oral administration of aureomycin. Two of the 4 cases developed pulmonary infections and the other 2 developed bacteremia and urinary infections. One of these 4 cases died of empyema due to *Ps. aeruginosa*.

**Conclusion**

A general review of the literature concerning the effects of all the commonly used antimicrobial agents on the normal fecal flora of man, lower animals, and domestic birds has been presented. Tyrothricin is effective chiefly against the Gram-positive flora. Limited experience with this antibiotic indicates that it produces no appreciable alterations of the intestinal flora in mice and may be too toxic for oral administration in man. The poorly absorbed sulfonamides are only moderately effective and also have the following disadvantages:
(1) Their action is slow. (2) Large doses are necessary. (3) Certain intestinal flora such as Pseudomonas, Proteus, yeasts, and enterococci are refractory to them. (4) Toxicity from the drugs is always a possibility. Formosulfathiazole appears to be more effective especially in reducing the number of Proteus, streptococci and clostridia. More studies are needed to attain a better evaluation of this drug.

Penicillin has little or no effect in suppressing the predominating Gram-negative flora of the intestinal tract. Streptomycin acts more rapidly and is much more effective against the predominating Gram-negative flora than the sulfonamides, but unfortunately the rapid development of streptomycin-resistance by the Gram-negative organisms in the intestinal tract has greatly limited its usefulness. Neomycin possesses a strong and rapid bactericidal activity against a wide variety of both Gram-positive and Gram-negative organisms, and has been considered by Poth (88) and Riddell (123) as the most effective intestinal antiseptic yet discovered. However, it has the disadvantage in occasional failure to inhibit the growth of Aerobacter. Bacitracin is of little value. Polymyxin B is very active against many Gram-negative bacilli, especially Ps. aeruginosa and the streptomycin-resistant coliforms. Drug-fastness does not occur readily. Unfortunately, most Gram-positive organisms, Proteus and yeasts are resistant to this agent. Among the broad-spectrum antibiotics chloromycetin is not generally considered to be effective in significantly reducing the intestinal flora. Early reports on aureomycin and terramycin indicated that these antibiotics are very effective, because they could produce rapid reduction of a great variety of the intestinal flora. Conflicting
reports concerning their real effects soon appeared. The current view inclines to believe that these broad-spectrum antibiotics are not suitable for preoperative use to suppress the intestinal flora, because the medication may actually cause a net increase in the total bacterial count due to overgrowth of the insusceptible Proteus, staphylococci, yeasts, Pseudomonas, and enterococci. Many serious complications as a result of this alteration have been illustrated. Combined therapy may prove to be more effective than any single component in many instances. After all, the ideal antimicrobial agent for preoperative use as an intestinal antiseptic is yet to be discovered.
MATERIALS AND METHODS

Selection of Experimental Subjects and Dosage of Medication

Fourteen normal healthy subjects of both sexes were selected who preferably never had received aureomycin or terramycin in the past and had not received antimicrobial agents of any kind shortly before the investigation. These subjects were each given aureomycin, 250 mg orally four times daily (1 g per day) for 5 days. Two of these subjects were studied twice. The free interval between the 2 courses of medication was 8½ months in one case (W.L.) and 10 days in the other (E.B.) who received the medication for 3 days only during his first period of investigation. The investigation was also made on 2 additional aureomycin subjects through the courtesy of Dr. Maxwell Finland of the Boston City Hospital. One of these (W.T.) was given 2.5 g of the drug orally daily for 8½ days and the other (J.P.) 20 mg orally daily for 15 days, then 1 g intravenously daily for 6 days and finally 1 g orally daily for 6 days with a free interval of 10 to 14 days between each 2 periods of medication. For comparison, three terramycin subjects (V.P., M.P. and R.F.) were also studied through the courtesy of Dr. Henry Bakst of the Boston University Medical School. These were aged 7, 10 and 12 and were each given 200 mg of terramycin orally four times daily for 5 days to treat their enterobiasis.

Bacteriological Studies

Fecal specimens were studied daily in a great majority of the cases for a period of 5 days before medication in order to obtain a basic picture of the fecal flora in each case, during the course of medication and following the medication for a varying number of days (up to 25 days).
until the fecal flora had returned to normal. The specimens were collected in clean wax-coated cardboard containers and were studied immediately. If delay was necessary, the specimens were immediately refrigerated. A sample totaling one gram was taken from several portions of each fecal specimen and was emulsified in 9.0 ml of sterile distilled water. The mixture was then blended into a homogeneous suspension with sterile glass rods. This suspension was diluted in sterile distilled water according to the technic approved by the A.P.H.A. for water analysis (122). The total aerobes were enumerated in pour plates of B.B.L. eugonagar using a 1.0-ml inoculum for each plate. The total anaerobes (including facultatives) were enumerated with B.B.L. anaerobic agar and Brewer anaerobic culture dishes, using a 0.1-ml inoculum for surface streaking. The coliforms were enumerated in Difco Violet Red Bile Agar using a 0.5 ml or 1.0 ml inoculum for making pour plates. The yeasts were enumerated on grape juice agar using 0.2 ml inocula for surface streaking. The grape juice agar was prepared by suspending 40 g of Difco Bacto-Agar in 750 ml of distilled water to which 250 ml of commercial grape juice had been added. This was then mixed thoroughly and boiled gently for 3 to 5 minutes, cooled and poured into Petri dishes. The medium contains approximately 5 per cent of sugar and 4 per cent of agar, and has a final pH about 3.9. In the writer's experience, it exerts no inhibition on the growth of yeasts and appears to be very selective, allowing only the growth of yeasts and other fungi probably due to its low pH. The staphylococci were enumerated on Difco Staphylo-
coccus Medium using 0.2 ml inoculum for surface streaking, and the organisms so obtained were confirmed each time with Gram stain smear and
catalase test. Representative strains of *Staphylococcus aureus* were saved for cultural tests for pathogenicity. *Streptococcus faecalis* was detected using Difco *SF* Medium containing sodium azide. Five tubes of the liquid medium were inoculated with each appropriate dilution and incubated at 45.5°C for 48 hours. When no growth occurred in any of the tubes after two days' incubation, the tubes were re-incubated for an additional 24 hours. From the number of positive tubes, the most probable number of *Str. faecalis* in each specimen was determined from the tables of Prescott (100). *Proteus* and *Pseudomonas*, when present, were enumerated on Difco *SS* Medium using 0.2 ml inoculum for surface streaking. All the plates were incubated aerobically at 37°C for 48 hours, followed by 24 hours' incubation at room temperature before final counting. The coliforms were usually counted at the end of the first 24 hours' incubation and the total anaerobes enumerated at the end of 48 hours' incubation. All the plates mentioned above were made in duplicate and the final count for each kind of organism is an average of the numbers obtained from every 2 plates. The counting was sometimes done with the aid of a hand magnifying lens, especially when the colonies were too small to be identified with naked eye.

No further identifications of the total aerobes, the total anaerobes and the coliforms were intended. However, the coliforms from the last 4 aureomycin subjects (A.P., A.C., K.C. and T.S.) have been identified and tested for their sensitivities toward aureomycin as a part of a separate research project (126). The results will not appear in this report. The yeasts were further studied for the presence of pseudomycelia and chlamydoc- spores in corn meal agar (18) after 3-7 days' incubation at room temperature.
The strains of the *Staph. aureus* were further studied for their ability to produce coagulase as well as to hemolyze blood. *Str. faecalis* was sometimes studied with Gram stain smear for its purity but no further identification was conducted. *Proteus* was first transferred to Kligler Iron Agar for observation on its ability to swarm and its preliminary sugar reactions. The species of the organism was then identified according to the following criteria, modified after Proom and WoIwood (99):

<table>
<thead>
<tr>
<th>Species</th>
<th>Fermentation of Glucose</th>
<th>Lact.</th>
<th>Mannitol</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>Indole</th>
<th>Prod. of $H_2$</th>
<th>Urea Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vulgaris</em></td>
<td>AG</td>
<td>—</td>
<td>—</td>
<td>AG</td>
<td>AG</td>
<td>/</td>
<td>/</td>
<td>rapid</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>AG</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>AG (late or neg.)</td>
<td>/</td>
<td>/</td>
<td>rapid</td>
</tr>
<tr>
<td><em>P. morganii</em></td>
<td>AG</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>/</td>
<td>/ (slow)</td>
<td></td>
</tr>
<tr>
<td><em>P. rettgeri</em></td>
<td>A</td>
<td>A</td>
<td>—</td>
<td>—</td>
<td>A (late or neg.)</td>
<td>/</td>
<td>/</td>
<td>rapid</td>
</tr>
</tbody>
</table>

*This species should be excluded from the genus *Proteus*, because of its inability to produce amines.

A---acid; G---gas.

All the bacterial counts were converted into numbers of organisms per gram of wet feces. The Logarithms of the numbers were finally plotted against time as the abscissa. The curves for each kind of organism were then divided into groups according to the patterns of alterations. Tabulation of results was done wherever indicated.

**Serological Studies**

Blood samples were taken before the administration of aureomycin and at intervals after cessation of the therapy in case of overgrowth of *Proteus*. The sera in each case were then tested against one strain of the organism isolated from the same subject during the peak of the overgrowth of the organism, using the usual tube technic for "O" agglutination test.
The antigen was prepared by boiling a water suspension of the organism for one hour. The titers of the agglutination were read usually after 18 hours' incubation at 50 C in a water bath.
RESULTS

Bacteriological Studies

A definite alteration in fecal flora was observed in all the cases following medication. Aureomycin and terramycin behaved in a similar manner, therefore the results for both drugs will be presented together. The data on the daily bacterial counts for each subject are presented as graphs (Figures 4 to 23). The duration of alterations in fecal flora is summarized in Table 1.

In one subject (J.P.) the intravenous administration of aureomycin seems to have caused less alteration in the fecal flora than the oral administration of the drug in the same daily dose. Observations on this single case do not permit any conclusions regarding differences due to routes of administration of the drug. In the same case, 20 mg of aureomycin orally per day for 15 days produced no appreciable alteration in the fecal flora except for a 100-fold increase in yeasts. The increase in numbers started on the second day, reached its peak on the 12th day of medication, and then the number of yeasts gradually decreased to the pre-drug level 5 days following discontinuance of the medication.

As to the degree of alteration due to difference in dosage of medication, again no definite conclusion can be drawn between the changes produced in subject W.T. given 2.5 g of aureomycin orally per day for 8 days and the other subjects given only one gram of aureomycin orally per day for 5 days. Generally the pattern of alterations in the fecal flora varies considerably from subject to subject as well as from organism to organism. The data presented below will tend to show the common patterns for each group of organisms studied. Some of the results of this
investigation have been previously reported (68).

1. Coliforms: In this investigation the term "Coliform" includes all lactose fermenting Enterobacteriaceae. The alteration in number of coliform organisms during and following medication is summarized in Table 2. The patterns of alteration are illustrated in Figure 1.

Figure 1

The curves in Figure 1 represent data obtained from individual subjects but similar data were obtained for other subjects. The pattern of alteration generally noted in 14 subjects was a decrease in numbers
quickly followed by an increase in coliforms. The maximal decrease usually occurred on the second or third day following the start of the medication (in about 60 per cent of cases). The maximal increase in population following the initial decrease occurred in a period from 2 to 21 days following discontinuance of medication. The average was about 6 days. Several minor variations in the pattern were also noted.

Two subjects (A.B., H.B.) showed a late maximal decrease which occurred after the medication had been discontinued in contrast to the early maximal decrease which occurred during medication in all the other cases. Four subjects (M.P., W.L.-II, M.S., J.B.) showed increases in numbers of coliforms to well above the pre-drug levels. The other 8 subjects showed increases in numbers of coliforms only to the pre-drug levels.

Another pattern of alteration was noted in 3 subjects (A.K., M.C., V.P.) in whom there was no appreciable change in coliform population throughout the course of study.

In one subject (K.C.) the medication resulted in complete disappearance of coliforms from stool cultures. These data are listed separately because similar observations have been reported by other investigators (20, 21, 78, 82).

It required a period of from 0 to 15 days (average about 5 days) following discontinuance of the medication for the numbers of coliform to return to their pre-drug levels.

2. Total aerobes and total anaerobes: The term "total aerobes" includes all organisms culturable on B.B.L. eugon-agar and in the case of "Anaerobes" includes all organisms culturable on B.B.L. anaerobic agar.
Generally these groups of organisms seem to follow the same patterns as do the coliforms. The decreases in numbers of both aerobes and anaerobes during medication were often much less marked and in no case were they eliminated completely. Clear-cut exceptions were noted in 2 subjects (T.S., D.L.) in whom the numbers of coliforms decreased while the numbers of total aerobes and total anaerobes showed only slight changes.

Periods of from 0 to 17 days following discontinuance of the medication were required for the numbers of total aerobes and total anaerobes to return to their pre-drug levels. The average was about 7 days.

3. Yeasts: Yeasts were found in 11 subjects or in approximately 60 per cent of the cases studied. 142 representative strains isolated from these 11 subjects were further studied on corn meal agar. Strains developing both pseudomycelia and chlamydospores on this medium are generally considered to be Candida albicans (18). On this basis 109 strains (77 per cent) were considered to be C. albicans. Only C. albicans was isolated from 6 of the 11 subjects in whom yeasts had been found. The other 5 subjects had C. albicans and other unidentified yeasts.

In most instances, the yeasts showed a rapid 100-fold to 1,000-fold increase without undergoing any initial decrease in population during medication. A period from 0 to more than 15 days after the medication had been discontinued, with an average of 7.5 days, was required for the counts to return to their pre-drug levels. Four subjects (V.P., M.S., R.P., A.C.) who did not have detectable yeasts before medication showed the presence of and proliferation of yeasts during and following medication. Two subjects (E.B., R.M.) showed fluctuations but no significant increase in the yeast populations during and following the medication. The other 9
subjects all showed definite increases in numbers of yeasts in response to the medication.

4. **Staphylococci**: Staphylococci were studied in the feces of 18 subjects. Their responses to medication were, in general, less uniform than in the case of the other organisms studied. However, 3 major patterns of alteration were observed and are illustrated in Figure 2.

![Figure 2](image-url)

**Figure 2**

**PATTERNS OF ALTERATION IN STAPHYLOCOCCI**

- **LOG OF THE NO. OF STAPHYLOCOCCI/GM. OF WET FECES**
- **TIME IN DAYS**

- **MEDICATION**

- **Subject H.B.** - Representative of 3 Subjects
- **" A.P. "**  " 3 "
- **" R.M. "**  " 12 "
A pattern of alteration was noted in 3 subjects (H.B., A.B., V.P.) in whom the decrease or disappearance of staphylococci due to medication was quickly followed by an increase in the population up to or above pre-drug levels. There was a complete disappearance of the staphylococci while receiving medication in two of these subjects (H.B., A.B.). The other subject (V.P.) showed only a small decrease in numbers of staphylococci during medication.

In three subjects (A.P., K.C., D.L.) there were some fluctuations but no significant alterations in staphylococcal populations due to medication.

A 100-fold to 1,000-fold increase without any initial decrease in population due to medication was noted in 12 subjects (67 per cent of cases). Half of these subjects showed maximal increases in staphylococci during medication, and the other half showed maximal increases following discontinuance of the medication.

It took a period of from 2 to 15 days, or slightly longer, following cessation of medication for the number of staphylococci to return to their pre-drug levels for all subjects who had an increase in staphylococci.

Seven subjects (39 per cent) harbored staphylococci with golden-yellow colonies. Two of these subjects did not have such organisms until medication. In one case (J.P.) they were found during medication and in the other (M.C.) after discontinuation of medication. The other 5 subjects all showed the presence of golden-yellow staphylococci during the pre-drug period. Further study of representative strains isolated from the 7 subjects showed that the staphylococci isolated from 2 subjects (M.S., M.C.) were coagulase-negative but those isolated from the other 5 (M.P.,...
J.K., W.L.-II, J.B., K.C.) were hemolytic, coagulase-positive and mannitol-fermenting.

During medication there was an interesting shift between the staphylococci with golden-yellow colonies and the staphylococci with white colonies noticed in 4 subjects (M.S., J.B., W.L.-II and K.C.) receiving aureomycin and one subject (M.P.) receiving terramycin. The staphylococci with golden-yellow colonies were totally replaced by staphylococci with white colonies. In three subjects (M.P., M.S., W.L.-II) the latter proliferated rapidly towards the end of the medication period, and with cessation of medication, the staphylococci with golden-yellow colonies re-appeared at the time the population of the staphylococci with white colonies had started to decline. In another subject (J.B.) the staphylococci with golden-yellow colonies did not return for as long as 7 days following discontinuance of the medication (further fecal samples were not available).

Complications due to staphylococci did not develop in any of the subjects studied.

5. Streptococcus faecalis: Organisms which grew in Difco S F Medium were considered to be Str. faecalis. Further studies of occasional cultures gave results consistent with a diagnosis of Str. faecalis. The numbers of Str. faecalis were followed in twelve subjects who received aureomycin. Several patterns of alteration were noted.
In 8 subjects there was an increase of 100-fold to 10,000-fold in population without an initial decrease during medication. Four of these subjects (A.K., D.L., T.S., J.P.) had a maximal increase during medication and the other 4 subjects (A.C., J.B., K.C., A.B.) showed maximal increase in about 2 to 8 days following discontinuance of the medication.

Three subjects (A.P., W.L.-II, M.S.) showed only insignificant fluctuations in the numbers of \textit{Str. faecalis} throughout the whole course of study.
**Str. faecalis** disappeared completely from the feces of one subject (H.B.) shortly following medication.

It took from 0 to 17 days for the numbers of **Str. faecalis** to return to their initial pre-drug levels following cessation of the medication. The average was about 5 days.

6. **Proteus**: In this study all non-lactose fermenting colonies having black centers on SS Medium were considered to be **Proteus**. Representative colonies were specifically identified. **P. mirabilis** was found in 10 of the 19 subjects studied. No other species of **Proteus** was encountered. Nine subjects receiving aureomycin, from whom **Proteus** could not be isolated before medication, showed the presence of **P. mirabilis** on the second or third day of medication. In another subject receiving aureomycin, who was a **P. mirabilis** carrier, this organism disappeared on the fourth day of medication, followed by a re-appearance and a marked rise in numbers to above the initial pre-drug level. In almost all cases rapid proliferation of this organism to over $10^7$ organisms per gram of wet feces was noticed within four days following the appearance or re-appearance of the organism. It took a period of from 6 to 25 days following cessation of medication for the organism to disappear or to return to its initial pre-drug level.

7. **Pseudomonas aeruginosa**: The stools of one subject receiving aureomycin showed the presence of **Pseudomonas** on the third day of medication. It increased in numbers to $10^6$ organisms per gram of wet feces in 2 days, then began to decrease, and disappeared 5 days following cessation of medication. No ill effects from the rapid proliferation of this organism were observed. This species was not recovered from the stools of any
other subjects.

**Serological Studies**

Most investigators consider that an infection leads to a rise in the titer of antibodies directed against the infecting agent. In order to determine whether or not the marked increase in numbers of *Proteus* in the stools of some of the subjects is accompanied by infection, serological studies of such persons were conducted. Agglutination tests were carried out on the serum of 9 subjects all of whom showed marked alterations in numbers of fecal *Proteus* due to medication. Sera collected before and after treatment from each subject were employed. Only 2 subjects (M.S. and T.S.) showed increases in agglutination titers and these were slight. Sera collected from one subject (M.S.) showed a rise in the titer from 0 to 1:8 on the 10th post-drug day and 12 days following appearance of *Proteus* in his feces. The titer dropped to 1:4 on the 29th post-drug day and 15 days following disappearance of *Proteus* from his feces. This subject showed no clinical evidence of infection with *Proteus*. Serum from the other subject (T.S.) had a titer of 1:4 on the second day following appearance of *Proteus* in her feces while receiving medication. A control specimen of serum taken before medication was unfortunately not available. Three days following the appearance of *Proteus* in the feces she started to have symptoms of *Proteus* urethritis which lasted for about 10 days. The agglutinin titer of her serum increased to 1:6, 6 days following disappearance of *Proteus* from her feces and 18 days before disappearance of *Proteus* from her urine and urethral cultures. Sera from the remaining 7 subjects did not show the presence of detectable antibody at any time.
Untoward Reactions and Bacterial Complications
Following Medication

In general, the dosages of the drugs used were well tolerated. Stools, during and immediately following the medication, were generally bulky in quantity and loose in texture as described by Brown (15) and Rivera and Sborov (107). Approximately 75 per cent of subjects experienced "gas distension" and some increased frequency of bowel movements. These symptoms usually started from one to three days after initiating medication and persisted as long as 2 days following cessation of the medication. At no time was watery or bloody diarrhea noticed. During medication impaired appetite was complained of by 6 subjects, general weakness by 3 subjects, mild nausea by 3 subjects and some feeling of "motion-sickness" by one subject. Two and one-half gm of aureomycin per day given to one subject (W.T.) seemed to cause more nausea than a smaller dosage.

Rapid proliferation of Proteus, staphylococci, yeasts, Pseudomonas or other organisms were not associated with diarrhea or disseminated infections in any subjects studied. One female subject (T.S.), however, developed symptoms of urethritis during the time of rapid proliferation of P. mirabilis. The symptoms started 4 days after initiating aureomycin therapy and 3 days following the appearance of Proteus in her stools, almost coinciding with the time of maximal increase in fecal Proteus population. P. mirabilis, identical with the Proteus in her feces, was repeatedly isolated and was the predominant organism in the urethral discharge and first portions of her urine specimens. The organism was found to be completely resistant to aureomycin, but somewhat sensitive to
streptomycin on disc-sensitivity test. The symptoms lasted for about 10 days, but the organism did not disappear from her urine and urethral cultures until the 33rd post-drug day and 24 days following disappearance of Proteus from her feces. She received 8 grams of gantrisin, 1.6 million units of penicillin and 2 grams of dihydro-streptomycin during a 2 days' period 4 days after the complication had started.
DISCUSSION

It is evident from the results obtained in the present study that the pattern of alteration of the fecal flora of normal humans varies considerably from subject to subject and from organism to organism. Predictions cannot be made with certainty. Generally speaking, the alteration of the fecal flora during oral administration of aureomycin or terramycin as reported here are less marked than those reported by other investigators. This may, in part, be a reflection of a general trend in the direction of increased antibiotic resistance of the normal flora of humans as a result of the widespread use of antimicrobial agents (33, 38, 69). It is not the purpose of this presentation to give the impression that broad-spectrum antibiotics have no value whatsoever for pre-operative use as a means of producing intestinal antisepsis. One should, however, be aware of the possible complications and consequently understand the limitations in the use of these drugs. For instance, the secondary increase of some organisms during medication as reported here would indicate the need for a relatively short over-all course of medication. With the dosage used in this investigation, the maximum effect of the antibiotics might be expected only during the first 2 to 3 days of medication and one may possibly obtain an adverse effect beyond that time.

The increase in numbers of coliforms, total aerobes and total anaerobes during the medication, with or without an initial decrease in numbers found in this study agrees well with some of the findings of Ganey et al. (42), Morton et al. (81), Tyson et al. (27), Allen (2). However, more detailed data have been obtained in the present investiga-
tion. Similar increases in numbers of these organisms have been previously reported by many other investigators (28, 31, 43, 120) in studies of streptomycin and sulfonamides. The appearance and rapid increase in numbers of A. aerogenes to replace the sensitive strains of E. coli during medication as found in two (A.P., K.C.) of the four subjects of this series in a separate study (126) may be a factor to explain the secondary increase of the coliforms. The persistence of Aerobacter during medication had been previously observed by Sborov and his associates (111) in patients during treatment with aureomycin and by Spaulding and his associates (120) during treatment with streptomycin and sulfathalidine. The increase in numbers of coliforms is usually reflected in a similar increase in total aerobes and anaerobes (including facultatives) of which the coliforms form a major portion. Exception to this is quite evident in at least one subject (T.S.) whose coliforms showed a decrease toward the end of the medication while the number of her total aerobes and total anaerobes showed increases due to rapid proliferation of Proteus to above the coliform population.

The increase of staphylococci, yeasts, Str. faecalis and Pseudomonas as reported here is in agreement with practically all of the recent reports in the literature concerning the effects of these two antibiotics. An obvious difference in this investigation, however, would be the absence of serious complications due to these organisms in contrast to a number of cases of staphylococcal diarrhea, moniliasis and proctocolitis reported by many other investigators (22, 45, 49, 63, 139). The absence of serious complications here could be partly due to the small doses and short duration of the medication and partly due to high
resistance or the excellent nutritional status of the healthy subjects studied.

The growth of yeasts seems to be stimulated by the medication in a great majority of the cases either directly or indirectly through suppression of other organisms. The direct growth stimulating effect of aureomycin has been demonstrated recently in vitro by Pappenfort et al. (84) and Huppert and his associates (54) and the factor responsible for this stimulation cannot be identified with the antibiotic activity of the drug. The incidence of C. albicans in feces as reported here appears to be 2 to 4 times higher than the figures in the literature (77, 123).

The incidence of Proteus in feces has increased from about 5 per cent before medication to about 50 per cent following medication. The former figure is still comparable to the 10 per cent incidence found by Rustigian and Stuart (110) who used direct plating of fecal specimens. The 50 per cent figure is certainly unusually high possibly due to a growth stimulation by the medication either directly or indirectly through the suppression of other organisms. Of all the micro-organisms studied in this investigation, Proteus seemed to react most profoundly to the medication with a rapid and marked increase in population. These organisms did not disappear or return to their initial pre-drug level until from 6 to 25 days after the cessation of the medication. Despite the changes noted in 10 of the 19 subjects studied, no evidence whatsoever has been obtained to suggest that a marked increase in numbers of Proteus might be responsible for a dysentery-like diarrhea. T.S. is the only female subject who experienced a considerable increase in numbers
of Proteus in feces due to medication and also happened to be the only case of complication (urethritis) due to Proteus in this series. The 9 male subjects carrying Proteus developed no such complication under the same or similar circumstances. This discrepancy may be at least partly due to anatomical differences between the two sexes, since the external urethral orifice is located closer to the anal region and consequently is subjected to greater risk of fecal contamination in females than in males. Of course, difference in personal hygiene could be another factor in that regard. This experience indicates the need for a careful watch and prompt institution of suitable preventive measures for such complications in case of a marked increase of Proteus, especially in females. It is, therefore, suggested that in case of females Proteus should be watched for in the feces by careful routine stool cultures at least every other day during and shortly following medication. This routine stool culture can also provide information concerning alterations in other organisms.

The overgrowth of Proteus seemed to exert varying degrees of suppression on coliforms in 2 subjects (A.B., T.S.), on staphylococci in 3 subjects (T.S., A.P., W.T.) and on Str. faecalis in 2 subjects (W.L.-II, A.B.). These organisms showed an actual decrease or at least a suspension of their original tendency to increase while Proteus flourished. They returned to their original numbers after the Proteus had started to decline. Whether or not this is a matter of specific microbial antagonism or only the result of simple suppression due to overgrowth of one organism over the other remains to be investigated.

The increases in agglutinin titers in the sera of two subjects (M.S.)
and T.S.) suggest the possibility of *Proteus* infections. Infection, if any, in M.S. was subclinical in nature, whereas the urethritis in T.S. was clinically obvious. In the remaining 7 *Proteus*-carrying subjects studied serologically, the failure to develop an antibody response as demonstrated by agglutination tests may, at least, in part, be due to the lack of invasiveness of the organism and the resulting insufficient antigenic stimulation.

There are many reports in the literature which are in disagreement with the data presented here concerning changes in the fecal flora of man. Several factors may be responsible for these differences of opinion. Some of these are:

1. **Difference in dosage of the antimicrobial agents used:** Rivera and Shorov (107) and McVay (78) have clearly demonstrated that greater therapeutic doses can produce greater suppression of the intestinal flora than the smaller doses of the same antibiotic under the same experimental condition.

2. **Difference in level of the intestinal tract where the flora were isolated and studied:** Again in McVay's investigation (78) comparing the effects of aureomycin on fecal flora and on intestinal flora isolated through cecostomy and colostomy wounds, it is evident that organisms comprising the intestinal flora from higher levels were suppressed earlier and to a greater extent than those present in feces. This view is supported by the findings of Johansson et al. (60).

3. **Use of adjuvant pre-operative measures such as colonic irrigation, catharsis, and low residue diet to reduce the population of intestinal**
flora.

4. Difference in technic: To illustrate, the writer is in entire agreement with Metzger and his associates (75) who have expressed the opinion that discrepant results may be obtained with a simplified streak plate technic as compared with the serial dilution-pour plate technic. It is also the writer's experience that by using technics and media different from those of Dr. T. M. Gocke of the Boston City Hospital for daily study of the same fecal suspensions (10^-1 dilution) from the same test tubes, the results obtained were different from those of Dr. Gocke. Nevertheless, the general trends of alteration obtained by the two groups of investigators agree fairly well for a great majority of the flora studied.

5. Differences in response of the host as clinically observed, in degrees of sensitivity of the intestinal flora to the antimicrobial agent and in the environment: Morton and his associates (81) have clearly pointed out that bacteriological evidence does not always confirm the clinical results. The degree of sensitivity is not only an inherent factor of the intestinal flora itself, but could also be modified by any previous medications given to the host. In regard to the environment, any factor such as drug or concomitant infection capable of altering the thermodynamic environment and culture medium in the intestinal tract would complicate the alterations of the fecal flora.
SUMMARY

1. The alterations in fecal flora in 19 normal subjects, 16 of whom re­ceived aureomycin and 3 of whom received terramycin are described.

2. Generally, the responses of the fecal flora to medication vary consider­ably from subject to subject as well as from organism to organism. The responses of each group of fecal organisms are analyzed and a few representative patterns of alterations are illustrated in cases of coliforms, staphylococci and Str. faecalis. In the majority of cases, the coliforms, total aerobes and total anaerobes seemed to follow a similar course of alteration—decrease followed by a secondary increase in population during medication. Yeasts and Proteus were generally stimulated by the medication. Proteus seemed to give the most marked and persistent alteration. The staphylococci and Str. faecalis, in general, responded to medication in a less uniform manner.

3. Oral administration of these antibiotics by no means renders the intestinal tract sterile. The time limit for achieving a maximal effect from the administration of broad-spectrum antibiotics is stressed.

4. There is no evidence that a marked rise of Proteus causes a dysentery-like diarrhea. The only significant complication following medication noted in this investigation was the development of Proteus urethritis in one female subject.

5. Agglutination tests for Proteus were carried out in nine subjects who showed extensive growth of Proteus in their feces due to medication. Two of these subjects showed increases in agglutinin titers. The remaining 7 subjects failed to show such increases. Possible explanation for this failure is given.
6. Some important factors contributing to the conflicting reports in the literature on the effect of these antimicrobial agents are discussed.
APPENDIX

TABLE 1

Durations of Alterations in Fecal Flora Following Medication

<table>
<thead>
<tr>
<th>No.</th>
<th>Subjects</th>
<th>Approximate Number of Days Required by The Organisms to Return to Their Pre-drug Levels Following Cessation of Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>W.L.-I</td>
<td>W.L.-I (treated with aureomycin for 3 days only), J.P.-I (treated with 20 mg of aureomycin daily) and J.P.-II (treated with aureomycin intravenously) are not included in the above table.</td>
</tr>
<tr>
<td>2</td>
<td>E.B.-II</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>W.T.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>V.P.*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M.P.*</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>R.P.*</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R.M.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>J.P.-III</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>A.B.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>H.B.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M.S.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>W.L.-II</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>J.B.</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M.C.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>D.I.</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>A.R.</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>A.P.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>A.C.</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>K.C.</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>T.S.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>coliforms</th>
<th>total aerobes</th>
<th>yeasts</th>
<th>total Proteus</th>
<th>Ps. Str. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>range</td>
<td>0 to 15, 0 to 17, 0 to &gt;15, 0-&gt;15, 6-25, 5, 0 to 17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*terramycin subjects.  
** NS--not studied. 
***organisms appeared during or following the medication. 
- not found.
TABLE 2
Alterations in Coliforms During and Following Medication

<table>
<thead>
<tr>
<th>NO.</th>
<th>Subjects</th>
<th>Range of Pre-drug Counts*</th>
<th>Lowest Counts Count* When (Day)</th>
<th>Highest Counts Count* When (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>W.L.-I</td>
<td>Not studied</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>E.B.-II</td>
<td>Not studied</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>W.T.</td>
<td>6.9 x 10^6 to 3.7 x 10^7</td>
<td>5.0 x 10^6 2</td>
<td>2.3 x 10^8 6</td>
</tr>
<tr>
<td>4</td>
<td>V.P.**</td>
<td>1.1 x 10^6 to 1.9 x 10^6</td>
<td>No decrease</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M.P.**</td>
<td>5.0 x 10^6 to 2.0 x 10^6</td>
<td>9.0 x 10^6 2</td>
<td>1.4 x 10^9 8(A)</td>
</tr>
<tr>
<td>6</td>
<td>R.P.**</td>
<td>7.0 x 10^6 to 5.8 x 10^7</td>
<td>6.0 x 10^5 3</td>
<td>1.2 x 10^8 4(A)</td>
</tr>
<tr>
<td>7</td>
<td>R.M.</td>
<td>3.1 x 10^6 to 1.1 x 10^8</td>
<td>1.6 x 10^4 3</td>
<td>7.2 x 10^7 3(A)</td>
</tr>
<tr>
<td>8</td>
<td>J.P.-III</td>
<td>1.1 x 10^6 to 1.6 x 10^6</td>
<td>8.0 x 10^5 3</td>
<td>5.6 x 10^5 5(A)</td>
</tr>
<tr>
<td>9</td>
<td>A.B.</td>
<td>1.2 x 10^7 to 5.4 x 10^7</td>
<td>4.2 x 10^4 4(A)</td>
<td>4.6 x 10^7 10(A)</td>
</tr>
<tr>
<td>10</td>
<td>H.B.</td>
<td>4.0 x 10^6 to 3.7 x 10^7</td>
<td>9.0 x 10^5 1(A)</td>
<td>1.9 x 10^8 5(A)</td>
</tr>
<tr>
<td>11</td>
<td>M.S.</td>
<td>4.0 x 10^6 to 1.7 x 10^7</td>
<td>1.0 x 10^5 2</td>
<td>1.6 x 10^8 3(A)</td>
</tr>
<tr>
<td>12</td>
<td>W.L.-II</td>
<td>6.2 x 10^6 to 1.8 x 10^7</td>
<td>7.0 x 10^4 2</td>
<td>3.0 x 10^8 2(A)</td>
</tr>
<tr>
<td>13</td>
<td>J.B.</td>
<td>1.5 x 10^5 to 9.0 x 10^5</td>
<td>1.1 x 10^4 2</td>
<td>5.0 x 10^7 3(A)</td>
</tr>
<tr>
<td>14</td>
<td>M.C.</td>
<td>9.0 x 10^5 to 5.3 x 10^5</td>
<td>1.5 x 10^5 1</td>
<td>2.6 x 10^6 3(A)</td>
</tr>
<tr>
<td>15</td>
<td>D.L.</td>
<td>2.0 x 10^7 to 2.8 x 10^8</td>
<td>8.0 x 10^4 5</td>
<td>2.5 x 10^7 9(A)</td>
</tr>
<tr>
<td>16</td>
<td>A.K.</td>
<td>5.4 x 10^6 to 1.2 x 10^7</td>
<td>6.4 x 10^5 2</td>
<td>1.7 x 10^8 5(A)</td>
</tr>
<tr>
<td>17</td>
<td>A.P.</td>
<td>8.2 x 10^6 to 1.0 x 10^7</td>
<td>9.4 x 10^5 3</td>
<td>1.9 x 10^7 21(A)</td>
</tr>
<tr>
<td>18</td>
<td>A.C.</td>
<td>1.3 x 10^6 to 5.9 x 10^6</td>
<td>9.0 x 10^2 2</td>
<td>1.7 x 10^7 2(A)</td>
</tr>
<tr>
<td>19</td>
<td>K.C.</td>
<td>8.0 x 10^5 to 3.0 x 10^7</td>
<td>Not found 4 to 1(A)</td>
<td>2.5 x 10^8 4(A)</td>
</tr>
<tr>
<td>20</td>
<td>T.S.</td>
<td>2.1 x 10^6 to 2.1 x 10^7</td>
<td>4.4 x 10^4 4</td>
<td>4.8 x 10^7 4(A)</td>
</tr>
</tbody>
</table>

* Count = number of coliforms per gram of wet feces
** Terramycin subjects
(A) After cessation of the medication
### TABLE 3

Agglutination Tests for Proteus

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sera tested</th>
<th>Strains of Proteus Tested</th>
<th>Agglutinin titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:2</td>
</tr>
<tr>
<td>A.B.</td>
<td>AB-4-27(S)</td>
<td>AB-4-30(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>AB-5-14(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AB-5-29(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>H.E.</td>
<td>HB-4-28(S)</td>
<td>HB-4-23(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>HB-5-13(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HB-5-29(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>M.S.</td>
<td>MS-4-28(S)</td>
<td>MS-5-4(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>MS-5-13(S)</td>
<td>&quot;</td>
<td>[**]</td>
</tr>
<tr>
<td></td>
<td>MS-6-1(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>W.L.-II</td>
<td>WL-8-4(S)</td>
<td>WL-8-15(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>WL-8-16(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>WL-10-13(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>D.L.</td>
<td>DL-9-3(S)</td>
<td>DL-9-7(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>DL-9-17(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>M.C.</td>
<td>MC-8-29(S)</td>
<td>MC-9-9(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>MC-9-19(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>A.K.</td>
<td>AK-9-24(S)</td>
<td>AK-9-23(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>AK-9-30(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>A.P.</td>
<td>AP-10-13(S)</td>
<td>AP-10-16(P)</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>AP-11-4(S)</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>T.S.</td>
<td>TS-11-23(S)</td>
<td>(TS-11-30) (urethra)</td>
<td>[**]</td>
</tr>
<tr>
<td>&quot;</td>
<td>TS-12-9(S)</td>
<td>(TS-12-7) (urine)</td>
<td>[**]</td>
</tr>
</tbody>
</table>

The positive tubes were recorded as \[\], \[\], \[\], \[\], \[\] according to the degree of agglutination. 0—no agglutination.

The numbers used to name the strains of Proteus and sera designate the month and the day on which Proteus was isolated or the serum was collected.
Keys to Figures 4 to 23

△—△—△—△—△— △Total anaerobes

△—△—△—△—△— △Total aerobes

X———X———X———X———X — Coliforms

○——○——○——○——○——○ — Total staphylococci

○——○——○——○——○——○ — Staphylococci (white colony)

●——●——●——●——●——● — Staphylococci (golden yellow colony)

△—△—△—△—△—△—△——△—△—△—△—△ — Yeasts

○···········○····················· — Streptococcus faecalis

●——●——●——●——●——●——●——●——●——● — Proteus mirabilis

○——○——○——○——○——○——○——○——○——○ — Pseudomonas aeruginosa
FIGURE 6, W.T. (male, 38)

Aureomycin
2.5 gm/day for 8 1/2 days
FIGURE 13, H.B. (male, 23)

AUREOMYCIN
1 g/day for 5 days

LOG OF NUMBER OF ORGANISMS PER CM^2 OF WET FIZZ

DATE
20-28-25-20-15-10-5-0
FIGURE 23, T.S. (female, 26)

AUREOMYCIN
1 g/day for 5 days

 PENICILLIN, STEPHOMYCIN & GENTAMICIN

LOG OF NUMBER OF ORGANISMS PER GRAM OF MTT PEARLS

DATE

17/23/24/25/26/27/28/29/30/1/2/3/4/5/6/7/8
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A QUANTITATIVE STUDY OF THE ALTERATIONS IN THE FECAL FLORA OF MAN FOLLOWING ORAL ADMINISTRATION OF AUREOMYCIN

Abstract of a Dissertation

Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

BOSTON UNIVERSITY GRADUATE SCHOOL

by

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1954
Conflicting reports have appeared concerning the ability of broad-spectrum antibiotics to reduce the population of the intestinal flora. The purpose of this investigation has been to study in detail the alterations in fecal flora following oral administration of aureomycin (chlortetracycline) with the hope of attaining a better understanding of the changes.

Nineteen normal young adults were each given either aureomycin or terramycin, 250 mg, orally 4 times daily for 5 days in a great majority of the cases. In bacteriological studies, fecal specimens were studied daily for a period of 5 days before medication, during the five days' course of medication and following the medication until the fecal flora had returned to pre-drug levels. A sample totaling one gram was taken from several portions of each fecal specimen, blended into a homogeneous suspension in 9.0 ml of sterile distilled water, and diluted serially in sterile distilled water. The total aerobes were enumerated in pour plates of B.B.L. eugonagar. The total anaerobes (including facultatives) were counted with B.B.L. anaerobic agar and Brewer anaerobic culture dishes. The coliforms were enumerated in pour plates of Difco Violet Red Bile Agar. The yeasts were counted on grape juice agar. The numbers of staphylococci were determined on Difco Staphylococcus Medium and the organisms obtained were tentatively identified by Gram stain and Catalase test. Streptococcus faecalis was counted in Difco S F Medium. The tubes of broth were incubated at 45.5 C. for 48 hours and the most probable number of Str. faecalis in each specimen was then determined from the tables of Prescott. Proteus and Pseudomonas, when present, were enumerated on Difco SS medium. All cultures were incubated aerobically at 37 C. for
48 hours, followed by 24 hours' incubation at room temperature. The coliforms were usually counted at the end of the first 24 hours' incubation. Total aerobes, total anaerobes and coliforms were not identified further. The yeasts were further studied in corn meal agar for the formation of pseudomycelia and chlamydospores. Representative strains of Staphylococcus aureus were studied for their ability to produce coagulase and to hemolyze blood. Strains of Proteus were identified culturally. In serological studies, blood samples taken before medication and at intervals following medication if Proteus was present, The sera in each case were treated for agglutination against one strain of the organism isolated from the same subject.

A definite alteration in the fecal flora was observed in all subjects. Aureomycin and terramycin behaved in a similar manner. In one case, 20 mg of aureomycin orally per day for 15 days produced no appreciable alteration in the fecal flora except for a 100-fold increase in yeasts. In another case, a high dosage (2.5 grams aureomycin orally per day for 8½ days) produced no greater alteration than did the usual one gram per day. Generally the pattern of alterations in the fecal flora varied considerably from subject to subject and from organism to organism. The common patterns of alteration for each group of organisms studied are:

1. **Coliforms:** The pattern of alteration generally noted in 14 subjects was a decrease in numbers of coliforms quickly followed by an increase. The maximal decrease usually occurred on the second or third day following the start of the medication. The maximal increase in population following the initial decrease occurred in a period from 2 to 21 days following cessation of medication. The average was about 6 days. Another pattern
of alteration was noted in 3 subjects in whom there was no appreciable change in coliform population throughout the course of study. In one subject, the medication resulted in complete disappearance of coliforms for stool cultures. For all the subjects studied, it required a period of from 0 to 15 days following cessation of the medication for the number of coliforms to return to their pre-drug levels.

2. **Total aerobes and total anaerobes**: Generally, these organisms seemed to follow the same patterns as did the coliforms, but their decreases in numbers during medication was often less marked and in no case were they eliminated completely. These groups of micro-organisms returned to normal in from 0 to 17 days (average about 7 days) following cessation of medication.

3. **Yeast**: Yeasts were found in 11 subjects. Only *Candida albicans* was isolated from 6 of the 11 subjects. The remaining 5 subjects had *C. albicans* and other identified yeasts. Nine of these 11 subjects showed a rapid 100-fold to 1,000-fold increase without undergoing any initial decrease in yeast population during medication. A period of from 0 to more than 15 days after cessation of medication was required for the yeast counts to return to their pre-drug levels. In this group four subjects who did not have detectable yeasts before medication showed the presence of and proliferation of yeasts during and following medication. The remaining 2 of the 11 subjects showed fluctuations but no significant increase in the yeasts population during and following the medication.

4. **Staphylococci**: Staphylococci were studied in 18 cases. Their responses to medication were, in general, less uniform than in the case of the other organisms studied. However, three major patterns of alteration were observed. In 3 subjects the decrease or disappearance of staphylococci
due to medication was quickly followed by an increase in the population up to or above pre-drug levels. In another 3 subjects there were some fluctuations but no significant alterations in staphylococcal populations due to medication. A 100-fold to 1,000-fold increase without any initial decrease in population due to medication was noted in the remaining 12 subjects. It took a period of from 2 to 15 days, or slightly longer, following cessation of medication for the number of staphylococci to return to their pre-drug levels for all subjects who had an increase in staphylococci. Five of these 18 subjects harbored hemolytic and coagulase-positive Staph. aureus and 2 other subjects harbored coagulase negative Staph. aureus.

5. Streptococcus faecalis: In 8 subjects there was increase of 100-fold to 10,000-fold in population without an initial decrease during medication. Three subjects showed only insignificant fluctuations in the numbers of Str. faecalis throughout the course of study. In one subject Str. faecalis disappeared completely from his feces for a day or so shortly following medication. It took from 0 to 17 days for the numbers of Str. faecalis to return to their pre-drug levels following cessation of the medication.

6. Proteus: Proteus mirabilis was found in 10 of the 19 subjects studied. Nine subjects from whom Proteus could not be isolated before medication showed the presence of P. mirabilis on the second or third day of medication. In the remaining subject, a P. mirabilis carrier, this organism disappeared on the 4th day of medication, followed by a reappearance and a marked rise in numbers to above the initial pre-drug level. In almost all cases rapid proliferation of this organism to over $10^7$ organisms per gram of wet feces was noticed within 4 days following the appearance or reappearance of the organism. There is no evidence that a marked rise in Proteus causes
a dysentery-like diarrhea. It took a period of from 6 to 25 days following cessation of medication for the organism to disappear or to return to its initial pre-drug level.

7. **Pseudomonas aeruginosa**: The stools of only one subject showed the presence of *Pseudomonas* on the third day of medication. It increased in numbers to $10^6$ organisms per gram of wet feces in 2 days, then began to decrease, and finally disappeared 5 days following cessation of medication.

In this investigation with one exception the rapid proliferation of *Proteus*, *staphylococci*, yeasts, *Str. faecalis*, *Pseudomonas* and other organisms did not cause any complications. One female subject developed urethritis due to *P. mirabilis* while her fecal *Proteus* was rapidly increasing in number toward the end of medication. This organism, identical with the *Proteus* in her feces, was repeatedly isolated and was the predominant organism in the urethral discharge and first portions of her urine specimens. The organism did not disappear from her urethral cultures until the 33rd post-drug day and 24 days following disappearance of *Proteus* from her feces.

In the serological studies, only 2 of the *Proteus* carrying subjects showed slight increases in agglutinin titers. One is the subject who developed *Proteus* urethritis and the other subject did not experience any ill effect from the overgrowth of *Proteus* in his feces.

Generally speaking, the alterations of the fecal flora following oral streptomycin or terramycin as reported here are less marked than those reported by other investigators. Oral administration of these antibiotics by no means renders the intestinal tract sterile. The time limit for achieving a maximal effect from the medication is stressed. Factors contributing to differences in experimental results in regard to the effect of these antibiotics on the fecal flora are discussed. Also given is an extensive
review of the literature concerning the alterations in intestinal flora of man and animals treated with commonly used antimicrobial agents.
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