onstrate the close relationship of the gallbladder and the pancreas in a novel way, and suggest that the pancreas controls the tone of the gallbladder. The operative procedure deprives the duodenum of the external secretion of the pancreas and at the same time leads to atrophy of the organ. It is conceivable that lack of sodium bicarbonate and of the pancreatic enzymes in the duodenum may bring about an alteration in the hormonal control of the gallbladder. On the other hand, atrophy of the pancreas may lead to the deficiency of some principle from the pancreas that is essential to good gallbladder function.

After ligation and division, the pancreatic ducts have a marked tendency to recanalize or to establish collateral ducts. Since this occurred in 5 of the dogs, these processes evidently take place fairly readily. This group of unsuccessful operations provides evidence, however, that surgical manipulation of itself is not responsible for the failure of gallbladder visualization in the successfully operated animals. Presumably, the surgical trauma and degree of innervation to the gallbladder region is the same in both groups of animals.

As this work shows, there is always some uncertainty as to the operative result when the pancreatic ducts are tied and cut. Use of intravenous cholecystography, particularly with *o*-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid, appears to furnish a convenient way of establishing the status of the pancreas and the pancreatic ducts in operated dogs. Additional work will be required to determine the limitations of the method and whether it is valid in dogs after an extended period of time.

In some ways the experimental results parallel certain clinical observations. Elman(2) pointed out that the gallbladder may not visualize if intravenous cholecystography is carried out during episodes of acute pancreatitis. Since then this observation has been confirmed by Silvani and McCorkle(3). Comfort and associates(4) have added further clinical reports on the failure of the gallbladder to visualize during acute and chronic pancreatitis. It is evident from the several reports, however, that the clinical picture is not too well defined.

Additional work is in progress to clarify both the experimental and clinical aspects of the interrelationship of the pancreas and the gallbladder.

Summary. Studies on dogs show that successful ligation and division of the pancreatic ducts inhibits the visualization of the gallbladder by cholecystographic media whether administered orally or intravenously. Failure of visualization appears to be due to the development of physiological stasis of the viscus. The experiments demonstrate a close relationship of the two organs and suggest that the pancreas may control the tone of the gallbladder

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Depression of Fecal Urobilinogen by Aureomycin and by Terramycin.* (18711)

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Modification of the intestinal flora by drugs and the resultant effects on the nutrition and

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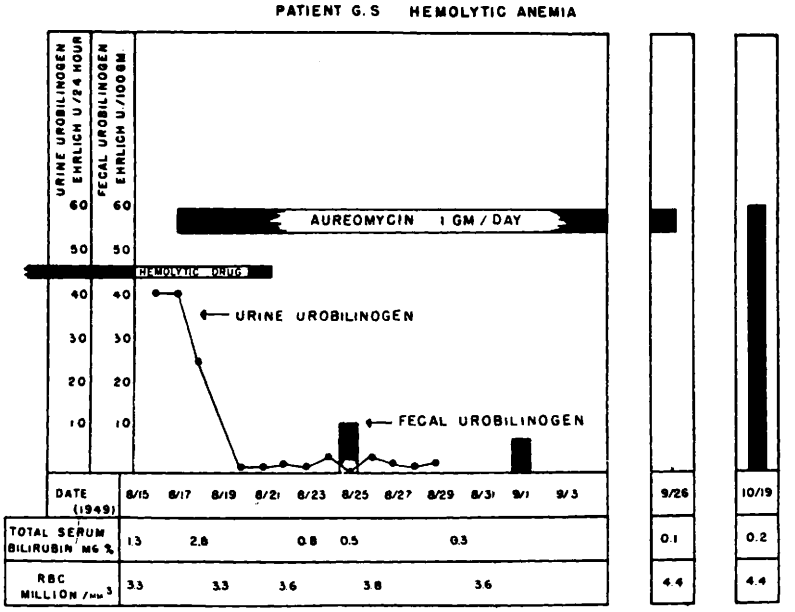

FIG. 1.

Changes in urinary and fecal urobilinogen concentration in a patient with hemolytic anemia who received aureomycin. Fecal urobilinogen concentration is indicated by the vertical bars.

metabolism of the host have received extensive study in experimental animals(1,2). It has been observed in man, also, that the administration of certain antimicrobial agents causes a decided alteration in the bacterial pattern of the intestinal contents(3).

It has been reported that the administration of aureomycin results in a marked reduction of urobilinogen in the feces of man, and that this change takes place concurrently with a reduction in the number of coliform bacteria in the feces(3c,d). It is the purpose of the present report to describe similar observations made in this laboratory on the fecal urobilinogen concentration of subjects who received aureomycin and terramycin.

Subjects and procedures. Patient G.S. was a 28-year-old white female with far advanced pulmonary tuberculosis and neuromyelitis optica who developed a hemolytic syndrome as the result of the administration of an experimental drug. Prior to aureomycin therapy the patient presented definite evidence of moderately severe hemolysis. The erythrocyte count was 3.3 million per cu mm, the serum bilirubin 1.3 mg per 100 ml, and the urine urobilinogen 40.5 Ehrlich units per 24 hours. Because of an intercurrent urinary tract in-

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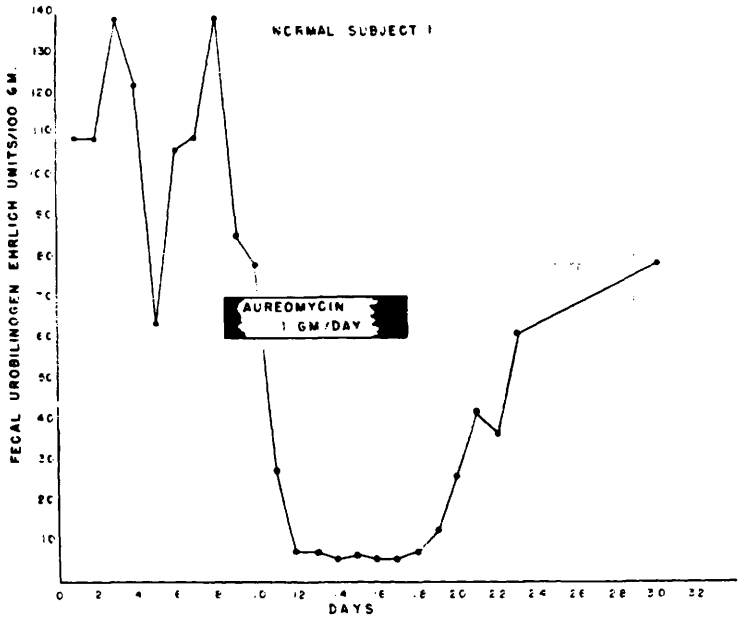

FIG. 2.
The fecal urobilinogen concentration of a normal subject who received aureomycin. Values are representative of those observed in 3 subjects.

fection the patient received aureomycin orally, 0.25 g every 6 hours, for 39 days (Fig. 1). Observations on the effect of aureomycin on fecal urobilinogen concentration were made in 3 normal persons. One subject received 1 g of aureomycin a day in divided doses for 9 days; one received 2 g a day for 5 days; and one received 2 g a day for 2 days. Observations on the effect of terramycin were made on stools from 7 patients receiving terramycin because of respiratory and urinary tract infections, but who were otherwise normal. Urine and fecal urobilinogen concentrations were measured by the method of Watson *et al.* (4) employing a Coleman junior spectrophotometer calibrated with Pontacyl dye standard. A wave length setting of 565 mμ was used. Twenty-four-hour urine volumes were measured on patient G.S. and urine urobilinogen excretion expressed in Ehrlich units per 24 hours (normal values range from 1 to 8 Ehrlich units per 24 hours). Fecal urobilinogen concentration was expressed in Ehrlich units per 100 g wet weight (normal values range from 40 to 160 Ehrlich units per 100 g). Urine specimens were kept stoppered and in the dark until tested. Stool specimens were stored in the refrigerator and were tested within 24 hours after collection.

Results. The observations made on the patient with hemolytic anemia (G.S.) are presented in Fig. 1. The urinary urobilinogen, decidedly elevated as a result of hemolysis, fell during the 72 hours following the initiation of aureomycin therapy to levels below 1 Ehrlich unit per 24 hours. It remained in this range, not exceeding 3 Ehrlich units per 24 hours, during the subsequent 9 days of aureomycin therapy during which measurements were made. Stool urobilinogen concentrations were measured on the 8th and 15th days of aureomycin therapy and were very low, being 13 and 8 Ehrlich units per 100 g, respectively. Although this patient's fecal urobilinogen concentration was not measured prior to aureomycin therapy, it is very probable that it was well above normal, in view of the frank hemolytic syndrome which she presented. Three weeks following the discontinuance of aureomycin, the patient's

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fecal urobilinogen concentration was normal.

The oral administration of aureomycin to the 3 normal subjects resulted, in each case, in a fall in stool urobilinogen to extremely low levels within 48 to 72 hours. The data presented in Fig. 2 are representative of the changes observed in all subjects. In all 3 subjects the range of fecal urobilinogen concentration after 48 to 72 hours of aureomycin administration was 4 to 8 Ehrlich units per 100 g.‡ One subject ingested the drug for only 48 hours, but this was sufficient to result in a sharp fall in the fecal urobilinogen from 100 to 6 Ehrlich units per 100 g.

After the discontinuance of aureomycin there was, in each subject, a period of 3 to 6 days during which pre-treatment fecal urobilinogen concentrations were gradually reestablished (Fig. 2). In no case was a normal concentration attained prior to 3 days after aureomycin.

The administration of terramycin was found, in a group of 7 patients, to produce a depression of fecal urobilinogen similar in all respects to that produced by aureomycin.

Discussion. In the present study it was observed that the urobilinogen content of the feces of a person ingesting aureomycin or terramycin diminished to a barely measurable concentration during the first 48 to 72 hours. It was maintained at this level throughout the duration of the treatment period, at least for as long as 14 days. Pre-treatment urobilinogen concentrations were usually restored within 6 days following the discontinuance of the drug.

The dynamics of this change in fecal urobilinogen content strongly suggest that it is the result of a distinct alteration in the intestinal flora. It is well known that other more obvious changes in the feces, indicative of altered flora, occur during aureomycin therapy. The stool becomes less solid, practically odorless, may change in color from a brown to a dark green, and sometimes has been observed to increase in bulk. The change in color may be reasonably explained as being due to the accumulation of bilirubin and its green oxidized derivative, biliverdin, due to the failure of the intestinal microorganisms to reduce bilirubin. The loss of the characteristic fecal odor apparently reflects the suppression of the putrefactive organisms in the intestine, which are capable of splitting proteins and of producing indole, skatole, and other amines responsible for fecal odor. The organisms believed most important in these putrefactive processes are the anaerobic bacilli and *E. coli*(7a).

The exact alterations in intestinal flora by which aureomycin or terramycin results in depression of the fecal urobilinogen have not been completely ascertained. Unfortunately, little is known regarding the precise nature of the organisms which reduce bilirubin to urobilinogen in the normal intestine. Kammerer and Miller (5) studied by *in vitro* methods, many of the organisms which normally inhabit the intestine and found that none, including *E. coli* and the obligate anaerobic spore-forming bacillus *B. putrificus*, was capable in pure culture of reducing bilirubin to urobilinogen. However, when bile was added to a mixed culture of *B. putrificus* and *E. coli*, the reduction was accomplished. Several aerobic species other than *E. coli* were capable of complementing the action of *B. putrificus* in this process. The presence of the anaerobic bacillus, however, was found essential to the reduction. These investigators concluded, therefore, that the reduction of bilirubin to urobilinogen *in vitro* was the result of the synergistic action of the obligate anaerobic bacillus, *B. putrificus*, and aerobic bacteria such as members of the coliform group. Furthermore, Kammerer and Miller linked the reduction of bilirubin with the formation of indole and the putrefactive processes, which are believed to be the result of the strong reducing properties of the anaerobes of the intestine(6).

Preliminary observations on the quantitative changes in the fecal flora during aureomycin or terramycin therapy made elsewhere

‡ These values represent density readings below .05 on the Coleman junior spectrophotometer. Because of the large error involved in readings in this range, they do not necessarily indicate the presence of any urobilinogen at all.

5. Kammerer, H., and Miller, K., *Deut. Arch. f. Klin. Med.*, 1923, v141, 318.

(3c,e-h), have indicated the most striking change in the *aerobic* portion of the flora to be a sharp decrease in the number of coliform bacilli concomitant with an increase in *B. proteus*. Apparently this is not the only change in bacterial pattern, however, as the loss of fecal odor would indicate the depression of those organisms which form odoriferous amines, *i.e.* the anaerobic bacilli.

On the basis of the present state of knowledge it would appear that the most acceptable hypothesis as to the mechanism of depression of fecal urobilinogen by antimicrobial agents, such as aureomycin or terramycin, is that it results from the elimination of, or marked reduction of, both the coliform bacilli and the putrefactive anaerobic bacilli in the bowel.

The practical clinical importance of the fact that some antimicrobial drugs depress fecal urobilinogen lies in the diagnosis of hemolytic anemias and the cause of jaundice. It is apparent that misleadingly low values would result in cases of hemolytic anemia, as in the case of patient G.S.; and in other cases of jaundice. Measurements of fecal urobilinogen are only truly representative of the amount of bile pigment in the intestine if the patient has not ingested aureomycin, or a similar antibacterial compound, within the preceding 6 or 7 days.

Summary. The oral administration of aureomycin or terramycin to man results in an extremely low concentration of fecal urobilinogen; a change which occurs during the first 48 to 72 hours of drug therapy, concurrently with other modifications in the character of the intestinal contents. Normal concentrations of urobilinogen may not be restored for so long as six or seven days following the discontinuance of drug administration. The depression of urobilinogen formation is probably the result of alterations in the flora of the intestine, which have not yet been completely defined. A case of hemolytic anemia is reported in which strikingly low fecal and urinary urobilinogen concentrations were observed while the patient was receiving aureomycin.

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In vitro and *in vivo* Studies of a New Antibiotic, Fumagillin, with *Endamoeba histolytica*.* (18712)

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Many antibiotics have been found to be effective both *in vitro* and *in vivo* against *Endamoeba histolytica*. For *in vitro* studies a culture of *E. histolytica* particularly appropriate for the determination of "direct amebacidal" action by agents which do not exert any effect on organisms growing in association with the ameba has recently been developed(1,2). A new antibiotic, designated "Antibiotic H-3" or fumagillin, has been kindly supplied to us for experimental trial by the Upjohn Co. and by Eli Lilly and Co. Fumagillin was first isolated from *Aspergillus fumigatus* by Eble and Hanson(3,4) in the Research Laboratories of The Upjohn Co.,

* Supported in part by the National Institutes of Health, Bethesda, Md., and the Abbott Laboratories, North Chicago, Ill.

† With assistance of Miss Dorothy Swanman.

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