(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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(Introduced by Hamilton H. Anderson)

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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.,

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† With assistance of Miss Dorothy Swanman.

1. Phillips, B. P., and Rees, C. W., *Am. J. Trop. Med.*, 1950, v30, 185.

2. Phillips, B. P., *Science*, 1950, v111, 8.

and McCowen, *et al.*(5) of the Lilly Laboratories were the first investigators to demonstrate its highly effective amebacidal properties *in vivo* and *in vitro*.

This paper is a report of studies undertaken to determine the *in vitro* effectiveness of this new antibiotic with some preliminary observations relative to the treatment of macaques infected with *E. histolytica*. Our investigations were based primarily on the initial studies by McCowen and his associates(5).

Materials and methods. The "amebacidal" end-point of fumagillin was determined by the procedure described in our earlier work (6,7). The culture of *E. histolytica* (F-22) associated with *Trypanosoma cruzi* (Culbertson) was obtained from Dr. C. W. Rees, National Institutes of Health, Bethesda, Md.(1). Since this antibiotic was found to be insoluble in water, 0.01 N NaOH was utilized as a solvent. The alkalinized solution of fumagillin was tested in serial dilutions against a 48-hour culture of *E. histolytica* growing in association with *T. cruzi*. The culture tubes were incubated at 37°C for 48 hours and examined. Subcultures were made from all the tubes in which amoebas were not demonstrated. To study the effect of this antibiotic on the trypanosomes, the "amebacidal" concentrations were tested against the trypanosomes alone. To determine the toxicity of the solvent 0.01 N NaOH, against the amoeba, controls were run with the alkali and no antibiotic. Chemotherapeutic studies were undertaken with two species of monkeys, *Macacus rhesus* and *M. philippinensis* naturally infected with *E. histolytica*. The majority of the animals had diarrheic stools before therapy. Their original weights varied from 1.5 to 4 kg. The monkeys were segregated in individual cages which were cleaned and scrubbed with lysol daily. The excreta dropped through wire floors on the bottom of the cages beyond the reach of the animals. Stool specimens were collected in the morning on clean paper towels placed on receiving pans under the wire floors. Fecal smears made promptly within 30 minutes after passage of the stools were fixed in Schaudinn's fluid. For food the monkeys were given cooked beans or wheat enriched by powdered milk, dry yeast and liver fraction L (Wilson Laboratories). In addition they received oranges daily and dog biscuits 2 or 3 times a week. The animals were irradiated with ultra-violet light for 1 to 2 hours daily. The fecal smears were stained by Heidenhain's iron-hematoxylin method. During the follow-up examination period after therapy 2 smears from each monkey were searched for protozoan parasites on 5 consecutive days every 2 weeks.

Results and discussion. The "amebacidal" level of this new antibiotic was found to be in dilutions from 1:10,000,000 to 1:15,000,000. The trypanosomes were not affected by these concentrations of the antibiotic. 0.01 N NaOH used as a solvent for the antibiotic was not toxic for the ameba. Fumagillin, or "Antibiotic H-3", was found to exert the most active *in vitro* effect of all the numerous antibiotics and other agents which have been tested in this laboratory against *E. histolytica*(6-9).

Fourteen monkeys were treated with fumagillin, each animal receiving from 50 to 125 mg/kg of the drug by mouth daily for 5 days. The results are presented in Table I. These preliminary therapeutic studies indicate that this new antibiotic is effective against *E. histolytica*. Follow-up examinations have disclosed that 4 of the 14 animals have shown *E. histolytica* in their stools following treatment with fumagillin. Monkeys No. 2, No. 3, No. 8 and No. 9 were found to be clear of amoebas at the end of 14 weeks. Monkey No. 6 died of tuberculosis at the end of one month

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TABLE I. Effect of Fumagillin Administered Orally on *Endamoeba histolytica* Occurring Naturally in Macaques.

Macacus rhesus or *M. philippinensis*	Dose of fumagillin, mg/kg	Duration of treatment, days	Recurrence of *E. histolytica* in stools after treatment
2	50	5	No recurrence after 14 wks
3	50	5	" " 14 "
6	50	5	" " 4 "
			Animal died of TB.
8	100	5	No recurrence after 14 wks
9	100	5	" " 14 "
S	100	5	Recurrence in 12 wks
M	100	5	" 10 "
11	100	5	" 10 "
R	100	5	" 4 "
R_1	125	5	No recurrence after 8 wks
S_1	125	5	" " 8 "
M_1	125	5	" " 8 "
10	125	5	" " 8 "
11_1	125	5	" " 8 "

following therapy; however, during this post-treatment period there was no recurrence of *E. histolytica* in the stools. Monkeys R_1, S_1, M_1, 10 and 11_1 showed no recurrence of *E. histolytica* at the end of 8 weeks.

The effect of fumagillin on non-pathogenic amebas could not be evaluated since only 3 monkeys had such amebas in their stools before the treatment for *E. histolytica* was instituted. However, it was found that 2 of these monkeys were cleared of *Endamoeba coli* during a 14 weeks' follow-up examination period. No toxic manifestations were observed in any of the monkeys treated with the antibiotic. The amount of hemoglobin and the number of erythrocytes and leucocytes remained within normal limits. Bromsulfalein tests indicated a possibility that a dose of 125 mg/kg might cause liver damage. In monkey S there was considerable retention of the dye. This animal has periodic bromsulfalein tests at the present time. Blood urea nitrogen showed an increase in only one monkey; this animal had received 100 mg of fumagillin per kilo of body weight. Electrocardiograms of all the animals were normal following therapy.

Summary. 1. A new antibiotic, fumagillin ("Antibiotic H-3"), was found to be effective *in vitro* against *E. histolytica* at concentrations of 1:10,000,000 to 1:15,000,000. 2. Preliminary *in vivo* studies on monkeys naturally infected with *E. histolytica* indicated that fumagillin possesses considerable amebacidal activity. Further investigations are necessary to evaluate this drug more completely.

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Effect of Sodium Bisulfite and Rutin on Action of Epinephrine. The "Bisulfite Phenomenon" in Man.* (18713)

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Richards(1) reported that sodium bisulfite increased the toxicity of epinephrine if these two compounds were injected subcutaneously or intramuscularly at the same site, but when given intravenously or injected at different sites no increase in toxicity, as measured by lethal doses, was noted. A review of the literature(2) as well as Richards' own studies showed that sodium bisulfite itself is relatively non-toxic. The U.S.P. permits the use of sodium bisulfite in concentrations up to 0.5% as an antioxidant in commercial epinephrine solutions, although not more than 0.2%

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† Jessie Horton Koessler Fellow.

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