sucrose solution of such concentration as to yield 60% sugar in a final volume of 30 ml. The prepared material was then fed in bottles with perforated screw caps to bees in wire cages, each of which contained about 100 recently emerged bees. Dead bees were removed daily and examined for the presence of cysts of the parasite in the epithelial cells of the ventriculus (*4*). Since the results of the first experiment with this substance were very promising, it was repeated with various modifications. The results of both experiments are summarized in Table 1. It is clear that

TABLE 1

INFLUENCE OF FUMAGILLIN ON NOSEMA DISEASE OF ADULT BEES

Treatments	Percentage dead bees with light or heavy Nosema infection after 17 days*
Expt 1	
1 Uninoculated	0
2 Inoculated	76
3 Inoculated + fumagillin (0.15 mg/30 ml)	38
4 Inoculated + fumagillin (0.75 mg/30 ml)	18
Expt 2	
1 Uninoculated	0
2 Inoculated	76
3 Inoculated + solvent for fumagillin in amount used in #5	73
4 Inoculated + fumagillin (0.5 mg/30 ml)	6
5 Inoculated + fumagillin (1.0 mg/30 ml)	2
6 Same as #5, but kept 2 days before feeding; cysts then centrifuged down, washed, and resuspended in sugar syrup	62

* Average of duplicates.

fumagillin caused a striking reduction in number of bees infected with *N. apis* and that this inhibition was not due to the action of the solvent. Furthermore, it appears that the cysts themselves are not affected by the antibiotic, as Treatment 6 in Expt 2 was designed to show, but that this compound probably exerts its effect when the cysts germinate. Since Nosema disease is most serious in overwintering colonies, the final test of the practicability of fumagillin in controlling the disease will have to be made with infected colonies maintained under these conditions. Such an experiment is being planned.

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The Treatment of Amebiasis with Fumagillin[1, 2, 3]

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The antibiotics now used in the treatment of amebiasis are believed to act primarily on the necessary bacterial associates of the amebae, thereby indirectly affecting the survival of the parasite (*1, 2*). Recently Hanson and Eble (*3*) have reported a new antibiotic, fumagillin, which has little antibacterial and antifungal activity. Subsequent *in vitro* experiments and animal studies by McCowen *et al.* (*4*) have shown this antibiotic to have marked amebicidal activity.

The present note reports our experiences with this antibiotic in adolescent and adult male patients who were hospitalized because of infection with the large race of *Endamoeba histolytica*. Of 22 patients treated in this series, 12 were asymptomatic, nine had symptoms of mild gastrointestinal irritation, and one had severe amebic dysentery. Fumagillin was administered orally in gelatin capsules to 18 patients for 14 days. Two patients received 5 mg daily; two, 5 mg twice daily; three, 10 mg twice daily; four, 35 mg daily in three divided doses; and seven, 50 mg daily in three divided doses. Four other patients were treated for 7 days. One received the 35-mg dosage, and three received the 50-mg dosage.

Laboratory studies on each patient consisted of frequent stool and urine examinations, stool and urine cultures, complete blood counts, blood urea nitrogen determinations, urea clearances, electrocardiography, and a battery of eight liver function tests, including prothrombin concentrations. Thiosulfate clearances were done on four patients, and in one patient the renal vein was catheterized and *p*-aminohippuric acid and creatinine extractions were performed. Evidence of therapeutic impairment of the hepatic, renal, or cardiovascular systems was not revealed by any of the clinical or laboratory procedures used in this study. Many of the patients had evidence of hepatic and renal involvement caused by schistosomiasis, but in none was the pre-existing disease aggravated.

Signs of toxicity were few and of little significance. Two patients receiving 50 mg daily complained of dizziness. In one this subsided while he was still receiving fumagillin, and in the other it subsided the day after the completion of therapy. Four other patients at this dosage complained of a loss of appetite without nausea or vomiting, but none lost weight during the period of treatment.

The disappearance of *E. histolytica* was prompt in

[1] A preliminary report.

[2] Fumagillin was supplied through the generosity of the Upjohn Company, Kalamazoo, Mich.

[3] This article has been released for publication by the Division of Publications of the United States Navy. The opinions and views set forth are those of the writers and are not to be construed as reflecting the policies of the Navy Department.

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the asymptomatic and mildly symptomatic patients. Stools became negative within 48 hr after the initiation of fumagillin treatment. In the two patients receiving 5 mg daily, the stools remained negative during treatment but became positive again within 5 days following the cessation of fumagillin therapy. One patient given 10 mg daily was positive again 6 weeks after the conclusion of treatment. This was the eighteenth post-treatment stool examination for this patient. All the other patients in this group have remained negative for *E. histolytica,* but none has been followed for more than 2 months, and some have been studied for only 3 weeks. Average number of post-treatment stools examined from each patient is 10.

The one patient with severe amebic dysentery did not respond as did the other patients. He was given 50 mg of fumagillin daily in three divided doses for 14 days, but stools which consisted almost entirely of blood and mucus on admission remained unchanged throughout treatment. During the last 5 days of treatment a sulfonamide was also administered without effect. Stools finally did become negative on the eighth day of treatment but remained negative only until the first post-treatment day. On the thirteenth day of fumagillin administration the rectal temperature reached 101° and there were signs of a hepatic mass. This mass has subsequently disappeared on treatment for an amebic abscess.

Other protozoan parasites observed in this group were *E. coli, Giardia lamblia, Chilomastix mesnili, Endolimax nana, Iodameba butschlii, Trichomonas hominis,* and *Plasmodium vivax.* All the enteric protozoa in this group were affected by the antibiotic, disappearing from the stools within 48 hr. Each, however, has recurred in at least one patient subsequent to the termination of therapy. Four patients had benign tertian malaria which became clinically apparent while under treatment, indicating the ineffectiveness of fumagillin in this infection. Other parasites present in these patients were *Schistosoma haematobium, Ascaris lumbricoides, Ancylostoma duodenale, Enterobius vermicularis,* and *Hymenolepis nana.* There was no indication of activity against any of these organisms.

The evidence thus far obtained in this study indicates that fumagillin is essentially nontoxic when given orally in dosages up to 50 mg daily for 2 weeks. It shows activity against at least 7 enteric protozoan parasites, being most effective against *E. histolytica.* Whether it will prove of definite value in the treatment of amebiasis must await further clinical trials with follow-up studies over several months. Its ineffectiveness in cases with deep amebic ulcerations is suggested.

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Kinetics of Reaction of Certain Vitamin B_{12} Analogs with Cyanide Ion

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The existence of several biologically active analogs of vitamin B_{12} has been recognized. These differ from each other in the nature of a coordinated anion, and are capable of interconversion under the proper conditions. Among the analogs so far reported, cyanocobalamin is outstanding in stability, and is formed from the others by treatment in water solution with cyanide ion (*1*). We have found that hydroxocobalamin (vitamin B_{12a}) can be titrated amperometrically with cyanide ion to a sharp end point in buffers of pH 8 or above; however, the rate at which the reaction occurs is inversely pH-dependent. The following describes some quantitative studies into the rate of reaction of cyanide ion with hydroxo- and thiocyanatocobalamin.

Reactions were carried out in 0.1 *M* borate buffers at 25°, cyanide ion concentrations being determined polarographically as described in a previous publication (*2*). The cell was charged with a solution of the appropriate cobalamin, and after purging with nitrogen for 5 min, an equimolecular amount of sodium cyanide dissolved in buffer was fed in from a syringe microburette. The recorder was then set in operation, giving in effect a graph of cyanide ion concentration versus time. Hydroxocobalamin and thiocyanatocobalamin were recrystallized preparations obtained as described by Kaczka (*3*) and Buhs (*4*). Their precise

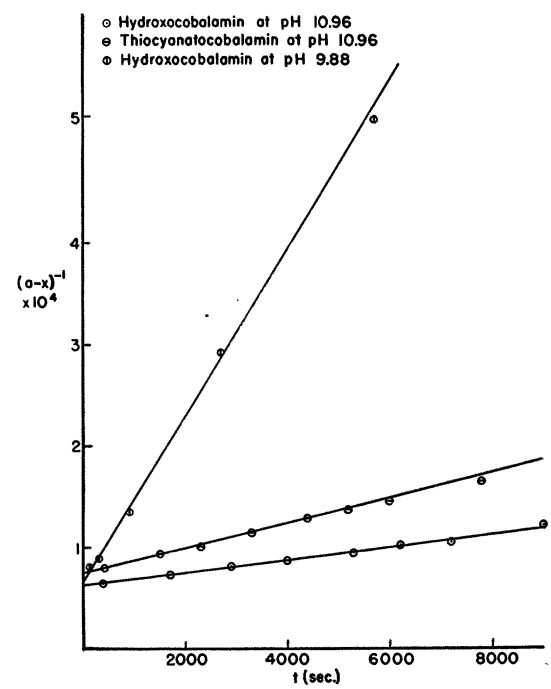

FIG. 1.