the nucleate on the enzyme, using a series of low substrate concentrations—in the range where an increase in substrate concentration causes an increase in enzyme activity—the nucleate concentration being smaller than that causing full inhibition. From Fig. 1 it is seen that if the reciprocals of the enzyme activities ($1/V$) are plotted against the reciprocals of substrate concentrations ($1/S$) a typical competitive curve is obtained, the nucleate concentration for this series being 0.75 mg/ml reaction mixture. It was postulated that the nucleate inhibition of arginase activity was caused by the removal by the nucleate of the Mn^{++} from the enzyme-Mn^{++} complex, thus rendering the enzyme inactive. The ease of inactivation of arginase by removal (by dialysis) of the activating Mn^{++} has been demonstrated by Mohamed and Greenberg (*1*). Furthermore, Neuberg and Roberts (*5*) have shown the ease of binding of Mn^{++} by sodium nucleate. According to this hypothesis, addition of more Mn^{++}—more than sufficient to saturate the binding capacity of the sodium nucleate—should restore the enzyme activity. It was found that addition of $MnCl_2 \cdot 4H_2O$ in quantities to give 0.025 mg added $MnCl_2 \cdot 4H_2O$/ml of reaction mixture (beyond the required Mn^{++} added initially for activating the enzyme) was sufficient to remove the inhibition by the nucleate.

It was further observed that enzyme inhibition is present only if the nucleate is mixed first with the enzyme portion prior to the addition of the substrate, but no inhibition takes place if the nucleate is mixed first with the substrate prior to the addition of the enzyme. The only explanation that might be given would be that the nucleate, after binding the arginine upon mixing with the substrate buffer, is incapable of removing the Mn^{++} from the enzyme-Mn^{++} complex. That sodium nucleate is capable of binding arginine was shown by Neuberg and Roberts (*5*). This interpretation also requires the assumption that arginase is capable of splitting off urea from the arginine-nucleate complex. Such a possibility would seem to be indicated from arginase specificity studies of Akasi (*6*), Boulanger and Bertrand (*7*), and Calvery and Block (*8*), who showed that if arginine is bound through the alpha NH_2 group with its COOH group free, the exposed guanidine group would be available for hydrolysis by arginase. Furthermore, since the substrate concentration employed was not in excess, there would have been a drop in enzyme activity if only those arginine molecules were available for hydrolysis which were not bound by the nucleate. However, since no drop in activity occurred, it is assumed that those arginine molecules which were bound by the nucleate were also available for hydrolysis by the enzyme.

Thus, since sodium nucleate causes a competitive type of arginase inhibition, and since the addition of Mn^{++} removed this inhibition, it is assumed that the nucleate causes enzyme inhibition by competing with the arginase molecule for the Mn^{++}. The absence of this inhibition upon mixing of the nucleate first with the substrate is explained as being due to the saturation of the nucleate binding capacity with arginine. It might further be postulated that nucleic acids, because of their enormous binding capacity, exist in the living cell in a form capable of either picking up or giving off enzyme-activating ions, depending on certain chemical changes in the cellular environment. In doing that, nucleic acids might conceivably be involved in the regulation of enzyme action inside the cell. It might be desirable to determine whether sodium nucleate would similarly inhibit *in vitro* other intracellular enzymes which require activating ions that are easily bound by nucleates.

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Control of Nosema Disease of Honeybees with Fumagillin[1]

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Nosema disease is an infectious disease of adult honeybees caused by the protozoan *Nosema apis* (*1*). It is widespread, and under favorable conditions may cause extensive losses of adult workers and queens in the winter or spring. Attempts to control it with antibiotics, sulfa drugs, arsenicals, and antiprotozoan agents have so far proved unsuccessful (*2–6*), although it was recently reported (*4*) that sulphaquinoxaline (0.2%) lowered the percentage of dead infected bees in cages by about 35%: however, one third of the dead bees were still infected. In view of these failures to control the disease, the announcement of the striking amebicidal action of a new antibiotic, fumagillin (*7*), aroused great interest. Accordingly, some of this material was obtained (through the courtesy of the Upjohn Company) and tested against *N. apis* infections.

The antibiotic was dissolved in methyl alcohol and diluted to a definite volume with water. The required dosage, together with 1 ml of inoculum, consisting of 35 million *N. apis* cysts per ml, was added to a

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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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