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

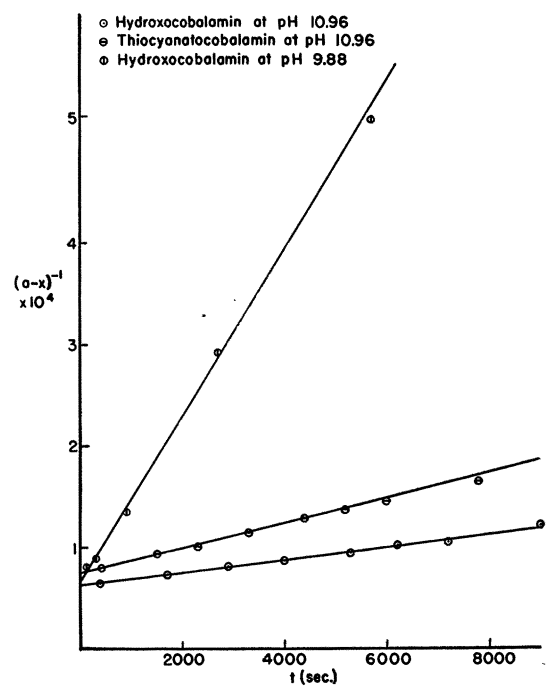

FIG. 1.

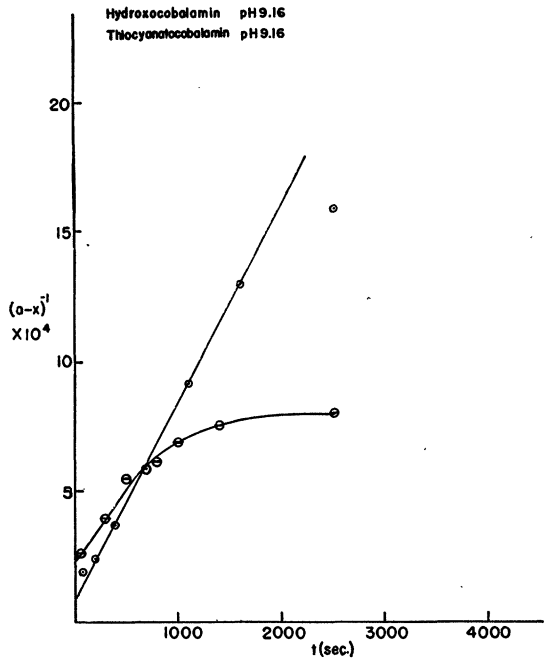

FIG. 2.

cyanide equivalents were determined by amperometric titration. Cyanide-binding impurities in the buffers were eliminated by adding to the buffer the amount of standard cyanide solution determined from a blank titration on an aliquot; the "cyanide-neutral" buffers were preserved in polyethylene bottles.

The reactants being in equimolecular proportions (about $1-2\times10^{-4}\ M$) the reaction rate law followed is

$$\frac{1}{a-x}=kt+\frac{1}{a},$$

where a is the initial concentration of cyanide ion, and $a-x$ the concentration remaining at time t. The data assembled are graphed in Figs. 1 and 2. From the slopes of the lines, the rate constants k and half-lives ($1/ak$) are obtained (Table 1).

In the case of hydroxocobalamin, it is to be noted that the rate constant of the cyanide-binding reaction decreases by roughly a factor of 10 for each unit increase in pH in the range studied. Since hydroxocobalamin is a pseudo base (*4*), whose basic properties are dependent on the hydrolytic reaction

$$\begin{bmatrix} - - OH \\ Co^{3+} \\ 0\ 0\ 0 \end{bmatrix}^{0} + H_2O \rightleftarrows \begin{bmatrix} - - H_2O \\ Co^{3+} \\ 0\ 0\ 0 \end{bmatrix}^{+} + OH^-,$$

the inverse rate dependence on pH is interpreted to mean that cyanide ion reacts not with hydroxocobalamin as such, but with the aquocobalamin ion in equilibrium with it (there being very much less of this ion present at pH 11 than at pH 9). It should be pointed out, however, that increase of reaction rate with diminished pH is limited by hydrolysis of cyanide ion, this becoming large below pH 9.

It is evident from Fig. 1 that the reaction between thiocyanatocobalamin and cyanide ion at pH 9.16 is not described by the rate law assumed above. The most probable explanation is that thiocyanate ion is not firmly bound by cobalamin at this pH, and that exchange with other ions in the solution occurs, giving rise to mixed cyanide reaction rates. The dissociation of thiocyanatocobalamin is clearly greater than that of the hydroxo analog at pH 10.96, since the half-life of cyanide exchange is only half as long.

These ion exchange reactions, accompanied perhaps by reversible reduction-oxidation of cobalt, are conceivably of importance in the biological function of vitamin B_{12}.

TABLE 1

RATE-CONSTANT DATA

Substrate	pH	$a\times10^4\ M$	Rate constant	Half-life (secs)
Hydroxocobalamin	9.16	0.86	73	120
"	9.88	1.63	7.9	850
"	10.96	1.58	0.63	9800
Thiocyanatocobalamin	10.96	1.64	1.30	4700

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Evidence of Authigenic and Detrital Glauconite

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Glauconite, the general formula (*1*) of which is $R_2'O,\ 4(R''O,\ R''_2O_3)10SiO_2\cdot4H_2O$, is a common constituent of many sedimentary rocks throughout the world, ranging in age from Cambrian to Recent. If a clear-cut distinction could be made between authigenic and detrital glauconite which would be universally applicable to glauconite-bearing sediments, a useful tool would be placed in the hands of the petrologist who is attempting to determine the origin of these sediments. Pettijohn (*2*) points out that "The distinction between autochthonous or primary glauconite, and transported or secondary glauconite, is seldom made either in recent or fossil deposits. Failure to make such a distinction has led to misconceptions concerning the genesis of glauconite."

The Upper Cretaceous, Eocene, and Pleistocene strata in the coastal plain of New Jersey afford an excellent opportunity for study of glauconite-bearing sediments. A systematic examination of glauconite grains from the several formations of this area reveals striking differences which make it possible to distinguish between authigenic and detrital glauconites.