of provoking vomiting, and relaxation of the cardia has been assumed to precede or accompany the vomiting act. These experiments indicate that the cardia is not easily influenced by alterations in pressure in a short isolated jejunal segment. However, relaxation of the cardia may occur in the dog with a moderate, localized jejunal distention without accompanying signs of nausea or other distress. The brief time interval between jejunal distention and relaxation of the cardia necessitates the assumption that this is a reflex. The pathways for the reflex have not been studied. However, the surgical procedure for preparing the jejunal fistula eliminates the possibility of this being an intrinsic reflex. If intrinsic pathways were present it is possible that they might contribute to the effect on the cardia.

It is possible, as in the case of the intestino-intestinal inhibitory reflex,[2] that this reflex could be elicited with less pressure if a longer segment of jejunum were distended. It is also quite possible that the cardia may be influenced more readily by duodenal than by jejunal distention.

Summary and Conclusions. Experiments were undertaken in unanesthetized dogs to study the effect of distention of a segment of jejunum, in the form of a Thiry fistula, upon the tonus of the cardia and upon the relaxation of the cardia resulting from swallowing or from esophageal distention. It was found that the cardia is not easily influenced by changes in jejunal pressure. However, repeated moderate distention of a short jejunal segment may produce a decrease in tonus of the cardia without evidences of nausea or other distress. Secondly, it was found that distention of a jejunal segment does not alter the relaxation-contraction pattern of the cardia which is produced by the swallowing act or by esophageal distention.

2 Peterson, C. G., and Youmans, W. B., *Am. J. Physiol.*, 1945, **143**, 407.

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Isolation of an Antibiotic Agent Derived from a *Phycomyces* Active *in vitro* Against *Trypanosoma equiperdum*.*†

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The more important antibiotic agents obtained from microorganisms have attracted attention largely because of their antibacterial properties. Any concomitant activity against fungi, actinomycetes, protozoa, or viruses has usually been observed only subsequently, and coincidental to the selective antibacterial effects. This is true, for example, of the antifungal action of streptothricin,[1] the inactivation of certain bacteriophages by streptomycin,[2] and the parasiticidal activity of tyrothricin.[3] Actinomycin and streptothricin prolonged the lives of mice infected with *Trypanosoma equiperdum*.[4] *Trichomonas vaginalis* has been killed *in vitro* by gram-

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1 Reilly, H. C., Schatz, A., and Waksman, S. A., *J. Bact.*, 1945, **49**, 585.

2 Jones, D., *J. Bact.*, 1945, **50**, 341.

3 Weinman, D., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 38.

4 Robinson, H. J., *Some Toxicological, Bacteriological, and Pharmacological Properties of Antibiotic Agents*, Thesis, Rutgers University, 1943.

TABLE I.
Trypanocidal Action of Active Fungus Filtrates.

Organism	Medium	Filtrate "trypanocidal" in dilution*
Dematium sp.	Czapek-Dox	>1:2 but <1:8
Penicillium luteum-purpurogenum	Trypanosome†	>1:2 but <1:8
Penicillium sp. No. 2	Czapek-Dox	>1:8
Penicillium sp. No. 10	Corn steep liquor	>1:2 but <1:8
Phycomyces sp.	Glucose-peptone	>1:2 but <1:8

* Organisms immobilized in 90-180 min at room temperature.
† *T. equiperdum* cells as the sole source of nitrogen.

icidin.[3] More recently, tyrothricin administered in a 95% alcohol solution has been reported to be curative against sporozoite- or blood-induced infections of *Plasmodium gallinaceum* in chickens.[5] Only a few agents, notably gliotoxin[6] and the *B. simplex*[7] factor, were studied largely because of their antifungal properties.

This report presents the results of a study begun in December 1943, with the purpose of isolating an antibiotic agent active against *Trypanosoma equiperdum*.

Despite the controversial status of specific soil enrichment as a technic for the isolation of antagonistic microorganisms,[8] it was decided to use this method of approach. Glass tumblers were filled about two-thirds full with a fertile field soil which had been sifted to remove coarser material, and which approximated a sandy loam in texture. Its pH was approximately 6.5. Throughout the experiments, the tumblers were incubated at 28°C, the moisture content of the soil maintained at a favorable level, and frequent mixing facilitated aeration.

Eleven enrichments of *T. equiperdum* were added to the soil, at irregular intervals, over a 3-month period. Each treatment consisted of about 1.0 g or less of trypanosomes concentrated by differential centrifugation from rat or mouse blood obtained as near the height of infection as possible. Since it was necessary to transport the protozoa from one laboratory to another, the sedimented organisms were frozen with dry ice. Consequently, only dead trypanosomes were added to the soil, and to the selective medium subsequently used for the isolation of antagonistic microorganisms.

After the fourth and again after the last enrichment, the soil was plated out on a medium consisting of 1.5% washed agar, 1% glucose, 0.05% K_2HPO_4, 0.05% KH_2PO_4, and distilled water. A suspension of sedimented *T. equiperdum* was added to this medium to supply nitrogen, in an amount sufficient to produce a definite turbidity. Plates prepared with this medium allowed the development of fungi and of a few bacteria. These organisms were isolated, and grown in different liquid media at 28°C for varying periods of time, after which the filtrates were tested for trypanocidal action *in vitro*.

When tested by an *in vitro* assay in which trypanosomes were exposed at room temperature to varying dilutions for 90-180 minutes, the non-sterile filtrates of 5 fungi were found to exhibit limited antiprotozoan activities (Table I) which could not be attributed to unfavorable pH. However, when the sterilized culture filtrates were left in contact with the organisms for 18 hours, the filtrate of one fungus, which was later found to be a *Phycomyces*, proved to be trypanocidal at much higher dilution, which varied markedly in individual experiments. Further studies were carried out with this organism, which appeared to belong either to the genus *Phytophthora* or *Pythium*.

The active factor was produced by this phycomyces in a glucose-peptone broth as

[5] Taliaferro, L. G., Coulston, F., and Silverman, M., *J. Inf. Dis.*, 1944, **75**, 179.

[6] Weindling, R., *Phytopath.*, 1943, **24**, 1153.

[7] Allen, M.C., and Haenseler, C. M., *Phytopath.*, 1935, **25**, 244.

[8] Waksman, S. A., and Schatz, A., *J. Bact.*, 1946, **51**, 305.

TABLE II.
Trypanocidal and Antibacterial Activity *In Vitro* of Known Antibiotic Agents in Comparison with Material Derived from *Phycomyces* sp.

Antibiotic*	Highest dilution of solution which inhibited		
	T. equiperdum	*E. coli*	*B. subtilis*
Chaetomin	1:64		1:10,000
Clavacin	1:16,600		1:100,000
Fumigacin	1:500		1:10,000
Streptomycin	<1:200	1:200	
Streptothricin	1:2	1:1,000	
Second antibiotic produced by *S. griseus*†	1:300		1:1,000,000
Phycomyces factor	1:100-1:1,000	<100	<100

* Crude concentrated preparations.

† The second antibiotic factor obtained from *S. griseus* is an alcohol soluble agent extracted with ether from the mycelium of the organism.

well as in a synthetic glucose-asparagin medium. It could be adsorbed on Norit and eluted with organic solvents. The agent was alcohol-soluble and water-soluble. Higher concentrations were obtained by extraction of the pellicles from 10- or 12-day-old cultures with pyridine or benzene. By this procedure extracts were obtained which varied widely in activity, but usually immobilized trypanosomes in 1:100-1:1000 dilution. The latter was approximately 500 times more active than the untreated culture filtrate. The phycomyces factor may, therefore, be a lipid, a small amount of which may saturate the aqueous culture fluid whereas most of the substance is retained within the mycelium.

Solutions of the phycomyces factor possessed slight, if any, action against *E. coli, B. subtilis, B. mycoides,* and *S. lutea.* A comparison of the trypanocidal and antibacterial action of this agent and some well-known antibiotics is presented in Table II. The latter were crude preparations, their activity being indicated by their effect upon specific bacteria. The selective effect of the phycomyces factor upon the protozoan is of interest.

In white mice, the toxicity of an alcoholic concentrate of the agent was no greater than that of the alcohol. Similarly, the substance exerted no demonstrable therapeutic action in mice infected with *T. equiperdum* and treated with maximal tolerated doses of the alcoholic solution. The absence of toxicity and efficacy indicate that the compound was probably present in low concentration.

Summary. Several soil fungi have been found to produce filtrates active against *Trypanosoma equiperdum.* From a *Phycomyces* sp. a lipid-like substance was isolated which immobilized the trypanosome *in vitro* but exerted no protective action against the experimental infection in mice.

No inhibitive agent has heretofore been obtained from this group of fungi. The selective action of the phycomyces factor against the trypanosome, but not against bacteria, is of particular interest.