it would seem reasonable to postulate the occurrence of higher blood CO levels in herbivorous animals consuming sizable quantities of green vegetation than in nonvegetarian animals.

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Nemin: a Morphogenic Substance Causing Trap Formation by Predaceous Fungi

Abstract. Broths in which the nematode *Neoaplectana glaseri* had developed axenically caused the mycelium of the predaceous fungus *Arthrobotrys conoides* to differentiate into traps. The active principle was extracted from worm-free culture filtrates and named "nemin." The identity of nemin remains to be established.

Recent reviews by Duddington (*1*) summarized our present knowledge of predaceous fungi. These remarkable microorganisms can capture and kill nematodes by means of traps formed in response to the presence of their prey. The fact that they do not form traps when grown in pure culture, but do so in the presence of nematodes, suggests that some morphogenic substance produced by the worms is responsible for differentiation of the fungus mycelium into traps. Evidence substantiating this was obtained by Comandon and De Fonbrune (*2*) and by Lawton (*3*), who demonstrated that water in which nematodes had been suspended induced trap formation. The list of nematode-free preparations capable of causing predaceous fungi to form traps has been extended to include various animal sera and tissue extracts (*4*). The active principle in water in which nematodes had been suspended was destroyed by boiling (*2*), whereas that in guinea pig serum was thermostable and not affected by alcohol (*5*). The nature of the substance or substances causing trap formation has not been determined.

Table 1. The ability of culture filtrates of *N. glaseri* to cause trap formation by the predaceous fungus *A. conoides*.

Filtrate dilution	Trap formation	
	Uninoculated medium	Inoculated medium
0	–	++
1/5	–	++++
1/10	–	++
1/50	–	+
1/100	–	+
1/200	–	–
1/400	–	–

The nematode *Neoaplectana glaseri* and the predaceous fungus *Arthrobotrys conoides* (*6*) were employed in the present investigations. *Neoaplectana glaseri* was cultivated axenically in meat infusion broth supplemented with raw liver extract. The composition of the medium and the method of culturing the nematode were described by Stoll (*7*). Nematode populations were measured by direct microscopic counts of appropriate dilutions of the broths. Worm-free preparations were obtained by double filtration of broth samples through sterilized filter paper (H. Reeve Angel No. 802) under aseptic conditions. The activity of filtrates was determined by a simple dilution assay. Aliquots of the culture filtrates were diluted quantitatively in a series of sterilized water blanks, and 1 ml of each dilution was added to the surface of petri dishes containing 20 ml of maize-meal agar on which *Arthrobotrys conoides* had developed for 4 days at 28°C. The plates were returned to the incubator and examined microscopically (× 100) for trap formation 24 and 48 hours after treatment. The extent to which various dilutions of the culture filtrates caused trap formation by the fungus was recorded. Activity is reported as dilution units, the reciprocal of the highest dilution of a culture filtrate that caused the fungus to form traps.

When a washed suspension of living nematodes was added to the surface of petri dishes on which *A. conoides* had developed, the mycelium differentiated, producing networks of hyphal loops in which numerous worms were captured and destroyed. No traps were formed on plates which did not receive nematodes.

The activity of a broth freed of nematodes and assayed by the procedures described is shown in Table 1. The culture was 9 months old when it was tested. It had been inoculated with approximately 100 worms and supported a population of 110×10^3 nematodes after 6 weeks' incubation. The culture filtrate caused *A. conoides* to form traps and contained at least 100 but less than 200 dilution units of activity. Since uninoculated broth was inactive, the results provided unequivocal evidence that a metabolic product of the nematode was responsible for the formation of traps by the fungus. "Nemin" is proposed as the name of the substance or substances that cause trap formation by predaceous fungi. The morphogenic effect of nemin is illustrated in Fig. 1. The nemin activity of a second 9-month-old culture of *Neoaplectana glaseri,* which had supported 28×10^3 worms after 6 weeks', and 2×10^3 worms after 7-months', incubation, was more than 10 but less than 50 dilution units. The numbers of traps on plates treated with undiluted culture filtrates were consistently less than those on plates treated with low dilutions (1/5, 1/10) of the active broths (Table 1). This suggests that there was a nemin concentration optimal for inducing trap formation, or that the culture filtrates contained a nemin inhibitor which was removed by dilution.

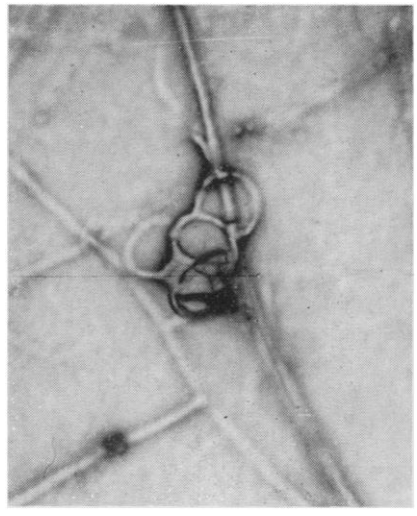

Fig. 1. Nematode-trapping hyphal loops produced by the predaceous fungus *A. conoides* in response to worm-free culture filtrates of the nematode *N. glasseri* (× 220).

To determine the time and stage of development at which nemin was elaborated by *N. glaseri,* a 100-worm inoculum was added to each of a series of tubes containing culture medium and the tubes were incubated at 22°C. Individual tubes were withdrawn after 4, 8, 12, 16, 21, 25, and 60 days of incubation. A portion of the contents of each tube was used to determine the number of worms present. The remaining broth was freed of nematodes by filtration and assayed for nemin activity. Following inoculation with third-stage larvae, young *N. glaseri* from the resulting adults first appeared on the fourth day, and the worm population then increased to a maximum in 15 days. The viable count decreased slightly after 21 days of incubation and then tended to remain con-

stant. The 60-day-old culture filtrate induced *Arthrobotrys conoides* to form traps. Broths of younger cultures were inactive, indicating that nemin was not produced during rapid growth and multiplication of the nematode but appeared in the medium after the nematode population had attained a maximum level and when death and disintegration of the worms had commenced.

Nemin is soluble in water, ethyl acetate, and *n*-butanol but not in benzene, carbon disulfide, or ethyl ether. It was not precipitated when culture filtrates were diluted to 5 times their original volume with acetone and was not inactivated by exposure to a temperature of 100°C for 10 minutes. The following procedure for the extraction and concentration of nemin was applied to an equal volume of human blood serum and the filtrate of a broth culture in which *Neoaplectana glaseri* had developed for 4 months: the fluids were diluted with four volumes of acetone and the precipitate formed was concentrated by centrifugation and discarded. The supernatant liquid was dried at room temperature under a hood, and the residue was dissolved in distilled water. The water was twice extracted with an equal volume of *n*-butanol, and the butanol extract was collected by means of a separating funnel and dried at room temperature. The residue was dissoved in distilled water and assayed for nemin activity. Both extracts induced trap formation, indicating that the active principle in serum and in a culture filtrate of *N. glaseri* was similar if not identical. Since the extraction procedure would have eliminated all protein and polysaccharides of high molecular weight, it is doubtful that nemin is related to antigenic materials excreted by some nematodes (*8*). The nature of nemin remains to be determined (*9*).

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Role of Myocardial Catecholamines in Cardiac Contractility

Abstract. In cats bilateral sympathectomy or administration of reserpine results in a marked reduction in concentration of myocardial catecholamines. The contractility of papillary muscles from such animals is significantly less than that of muscles from untreated animals. These findings demonstrate the importance of normal levels of myocardial catecholamines in the maintenance of normal cardiac contractility.

The capacity of reserpine to release norepinephrine and epinephrine from their storage sites provides another approach to the study of the role and mechanism of action of these neurohormones. Our recent experiments (*1*) showed that pretreatment of cats with reserpine, by depletion of the stores of catecholamines, abolished the positive inotropic responses of atropinized papillary muscles from these animals to tetramethylammonium, nicotine, and certain other ganglionic stimulants. Histological examination of the muscles has failed to reveal the presence of any ganglion cells (*2*). These observations indicate that the "nonganglionic" cardiac stimulant activity of tetramethylammonium and nicotine is dependent on the presence and release of catecholamines in the myocardium. Other workers (*3*) have also suggested that the augmented contractility of the myocardium which results from various procedures is due to intracardiac liberation of catecholamines. There is also experimental evidence which indicates that the heart rate of animals whose myocardial catecholamines have been depleted by pretreatment with reserpine is significantly slower than that of normal animals (*4*).

The present studies (*5*) were undertaken in order to determine the relationship of myocardial catecholamines to cardiac contractility. Papillary muscles of approximately equal length and thickness were prepared from cats according to the procedure described by Cattell and Gold (*6*). The muscles were subjected to a resting load of 2.0 g and were stimulated to contract by means of a square-wave stimulator which provided, at supramaximal voltage, 1 impulse per second with a duration of 1 msec. Their isotonic contractile amplitudes were recorded on a smoked drum by means of a lever providing tenfold magnification. After the contraction of the muscles had stabilized, the magnitude of contractile amplitude was measured. The myocardial content of catecholamines was determined spectrophotofluorometrically (*7*), and depletion of catecholamines was accomplished either by pretreatment with reserpine (*8*) or bilateral sympathectomy.

The mean values obtained for the contractile amplitude of papillary muscles and myocardial catecholamines from ten normal cats were 18.0 mm and 1.61 μg/g, respectively (Table 1). The intravenous injection of reserpine caused a marked depletion of myocardial catecholamines within 18 to 20 hours. The mean value in ten animals was approximately 90 percent below that found in untreated cats. The contractility of the papillary muscles from these cats was very weak compared with that of muscles from normal animals; the difference between the two groups was highly significant. It was also found that the papillary muscles from reserpine-treated cats were more readily fatigued than those from normal cats and at the same time were more sensitive to the inotropic effect of epinephrine or norepinephrine.

The afore-mentioned findings could be interpreted as being the result of a direct action of reserpine on the papillary muscle rather than a reduction in the concentration of myocardial catecholamines. To clarify this point similar experiments were performed on papillary muscles of cats whose myocardial catecholamines had been reduced in concentration by bilateral sympathectomy. Under pentobarbital anesthesia, bilateral removal of the stellate and first seven thoracic sympathetic ganglia was accomplished. Between removal of the ganglia on the two sides an interval of 7 to 10 days elapsed. Within 15 to 26 days after the last operation the myocardial catecholamines were found to be decreased by approximately 80 percent. The contractile amplitude of papillary muscles from these animals was depressed to about the same extent as that of muscles from reserpine-treated animals. It is to be noted that administration of reserpine resulted in a significantly greater reduction in cardiac catecholamines ($p < .001$) than did sympathectomy. Yet reduction in contractility was of approximately the same order of magnitude.

It can be concluded that depletion of myocardial catecholamines results in de-

Table 1. Myocardial catecholamine concentrations and contractile amplitudes of papillary muscles from normal, reserpine-treated, and bilaterally sympathectomized cats.

Treatment	Animals (No.)	Myocardial catecholamines (μg/g)*	Contractile amplitude (mm)*
None	10	1.61 ± 0.06	18.0 ± 1.10
Reserpine†	10	0.15 ± 0.03‡	9.7 ± 0.68‡
Bilateral sympathectomy§	9	0.28 ± 0.02‡	10.6 ± 1.05‡

* Mean ± standard error.
† Measured 18 to 20 hours after intravenous injection of 0.05 to 5.0 mg/kg of reserpine.
‡ These values are significantly different from control values ($p < .001$).
§ Measured 15 to 26 days after bilateral sympathectomy.