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*).

DAVID PRAMER
Department of Agricultural Microbiology, Rutgers University, New Brunswick, New Jersey
NORMAN R. STOLL
Rockefeller Institute, New York

References and Notes

1. C. L. Duddington, *Botan. Rev.* **27**, 377 (1955); in *Soil Zoology*, D. K. McE. Kevan, Ed. (Academic Press, New York, 1955), p. 284; *Biol. Revs. Cambridge Phil. Soc.* 31, 152 (1956).
2. J. Comandon and P. De Fonbrune, *Comp. rend. soc. biol.* 129, 619 (1938).
3. J. R. Lawton, *J. Exptl. Botany* 8, 50 (1957).
4. E. Roubaud and R. Deschiens, *Comp. rend.* 209, 77 (1939).
5. R. Deschiens and L. Lamy, *ibid.* 215, 450 (1942).
6. We are grateful to Dr. C. Drechsler for identification of the fungus.
7. N. R. Stoll, *J. Parasitol.* 39, 422 (1953).
8. P. B. Chipman, *ibid.* **43**, 593 (1957).
9. This report is a paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, the State University, New Brunswick, N.J. This investigation was supported in part by research grant G5949 from the National Science Foundation.

23 October 1958

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.

pression of cardiac contractility. It appears plausible also to conclude that the stores of norepinephrine or epinephrine in the myocardium are important in maintaining normal contractility. It is suggested that the myocardial catecholamines may be released in small quantities under normal conditions to affect the rate of the pacemaker and contractility and serve as humoral agents for the regulation of normal cardiac function.

Woo Choo Lee
F. E. Shideman

Department of Pharmacology and Toxicology, University of Wisconsin, Madison

References and Notes

1. W. C. Lee and F. E. Shideman, paper presented at the fall meeting of Am. Soc. for Pharmacol. and Exptl. Therap., Ann Arbor, Mich. (1958).
2. W. C. Lee, thesis, University of Wisconsin (1958); S. Middleton, C. Oberti, R. Prager, H. H. Middleton, *Acta Physiol. Latinoam.* 6, 82 (1956).
3. W. J. Whalen, N. Fishman, R. Erickson, *Am. J. Physiol.* 194, 573 (1958).
4. U. Trendelenburg and J. S. Gravenstein, *Science* 128, 901 (1958); J. H. Burn, *Brit. J. Anaesthesia* 30, 351 (1958).
5. This work was supported by a grant from the Wisconsin Heart Association.
6. M. Cattell and H. Gold, *J. Pharmacol. Exptl. Therap.* 62, 116 (1938).
7. P. A. Shore and J. S. Olin, *ibid.* 122, 295 (1958).
8. Reserpine (Serpasil) was generously supplied through the courtesy of A. J. Plummer, Ciba Pharmaceutical Products, Inc., Summit, N.J.

13 November 1958

Interference with Feedback Control: a Mechanism of Antimetabolite Action

Abstract. The action of an enzyme essential for tryptophan biosynthesis is inhibited by tryptophan and also by an analog of tryptophan. Similarly, histidine and one of its analogs inhibit the action of an enzyme essential for histidine biosynthesis. A mutant resistant to the histidine analog produces an apparently altered enzyme which is insensitive to both the analog and histidine.

Structural analogs of amino acids, purines, and pyrimidines have generally been considered to inhibit growth by interfering competitively with the *incorporation* of the corresponding normal metabolites into essential components of the cell. Evidence has now been obtained that analog action, in certain instances, may be explained by an alternative mechanism in which the analog inhibits the *biosynthesis* of the normal metabolite. Such a mechanism of analog action was suggested by recent observations concerning the control of biosynthetic reactions. Several metabolites, including valine (*1*), isoleucine (*1*), proline (*2*), and cytidine 5′-phosphate (*3*), have each been found to inhibit an enzymatic reaction necessary for its own biosynthesis. This type of inhibition constitutes a negative feedback system which permits the metabolite to regulate its biosynthesis (*1*). It was considered possible that an analog might act by mimicking the specific inhibitory effect of the corresponding metabolite.

This possibility has been confirmed by the finding that DL-6-fluorotryptophan (6-FT) (*4*), as well as tryptophan itself, is a potent inhibitor of the condensation of anthranilic acid with phosphoribosylpyrophosphate, a reaction essential (*5*) for the biosynthesis of tryptophan (Table 1). Similarly, histidine and its analog DL-2-thiazole alanine (2-TA) (*4*) are inhibitors of the synthesis of "compound III," an essential intermediate (*6*) in the biosynthesis of histidine (Table 2). DL-6-Fluorotryptophan is as effective as tryptophan for the inhibition of the condensation of anthranilic acid with phosphoribosylpyrophosphate. On the other hand, 20 times as much 2-TA as histidine is necessary for the inhibition of "compound III" synthesis. The difference in the effectiveness of the two analogs as enzyme inhibitors may be partially reflected in the difference in their bacteriostatic effects on *Escherichia coli* W. Colony diameter is reduced by 50 percent in the presence of $2 \times 10^{-7} M$ 6-FT, whereas 3500 times that amount of 2-TA is required for a similar reduction.

If such an inhibitory effect of an analog on the action of an enzyme necessary for the biosynthesis of the corresponding normal metabolite is indeed responsible for bacteriostasis, then the development of resistance might be accompanied by a decreased sensitivity of the affected enzyme to inhibition by the analog. An alteration of this type has been found in a mutant selected for resistance to $2 \times 10^{-2} M$ 2-TA. The enzymatic synthesis of "compound III" by extracts of this organism is completely insensitive to 2-TA (Table 2) (*7*).

In addition to this change, the enzyme in the mutant has also lost sensitivity to histidine (Table 2), and the organism excretes a compound provisionally identified as histidine. The parent strain, on the other hand, produces precisely enough histidine to meet its needs and does not excrete histidine. These observations provide evidence that the inhibition by histidine of the enzymatic synthesis of "compound III" is responsible for the precision of the feedback control of histidine biosynthesis in the parent strain. Cohen and Adelberg (*8, 9*) have reported excretion of other amino acids by mutants resistant to a variety of analogs, and it seems possible that the loss of feedback control which they postulate involves a mechanism similar to that described here.

The observations that both 6-FT and 2-TA mimic the specific inhibitory effects of their corresponding normal metabolites support a mechanism of analog action involving interference with the control of biosynthetic reactions. If the bacteriostatic effect of 2-TA on the parent strain is the result of such interference, as represented by the inhibition of the enzyme synthesizing the histidine precursor, "compound III," then in the mutant the insensitivity of this enzyme to inhibition by 2-TA accounts for the observed resistance. However, it is also possible that the bacteriostatic action of 2-TA is due to competition with histidine for incorporation into macromolecules. In this case the insensitivity of the enzyme to histidine, leading to the overproduction of this competitive metabolite, could account for the resistance of the mutant to 2-TA. It is hoped that

Table 1. Inhibition of the condensation of anthranilic acid (AA) with phosphoribosylpyrophosphate (PRPP) by tryptophan and by its analog, 6-FT. Phosphoribosylpyrophosphate was generated *in situ* from adenosine triphosphate and ribose-5-phosphate (PRPP kinase is present in excess in these extracts). The rate of condensation was determined according to the method of Yanofsky (*5*); 0.01 ml of an extract of *E. coli* W (12 mg of protein per milliliter) was used.

Inhibitor and concn. (M)	Inhibition of AA, PRPP condensation (%)
None	0*
L-tryptophan (5×10^{-5})	45
L-tryptophan (5×10^{-4})	62
6-FT (5×10^{-5})	40
6-FT (5×10^{-4})	80

* In the absence of an inhibitor 10.0 mμmole of anthranilic acid was metabolized in 10 minutes.

Table 2. Inhibition of "compound III" synthesis. The rate of synthesis was determined by a previously described method (*6*); 0.2 ml of extract (12 mg of protein per milliliter) was used. Extracts were prepared by sonic oscillation from a mutant resistant to DL-2-thiazole alanine (2-TA) and from the wild type, *E. coli* W.

Inhibitor and concn. (M)	Inhibition of "compound III" synthesis (%)	
	Wild type extract	Resistant mutant extract
None	0*	0*
L-Histidine (1×10^{-4})	59	< 1
L-Histidine (2×10^{-4})	73	< 1
L-Histidine (3×10^{-2})		< 1
2-TA (8×10^{-4})	28	< 1
2-TA (2×10^{-3})	57	< 1

* In the absence of an inhibitor 74 and 39 mμmole of "compound III" were synthesized in 15 minutes by extracts of the sensitive and resistant strains respectively.