MECHANISM OF ACTION OF PHENOMYCIN: COMPARATIVE STUDY WITH DIPHTHERIA TOXIN

Sir:

Phenomycin, a basic polypeptide antibiotic, exhibits significant inhibition of EHRLICH carcinoma, sarcoma 180, and adenocarcinoma 755 in mice but has no antimicrobial activity[1,2]. The mechanism of action of phenomycin has been studied[3]. The antibiotic inhibits protein synthesis to a significant extent in mammalian ribosomal systems, but much less in bacterial systems. The selective toxicity of the antibiotic is similar to that of diphtheria toxin. Moreover, both are simple proteins. The activity of phenomycin on protein synthesis was comparatively studied with diphtheria toxin. It has been observed that diphtheria toxin inhibits protein synthesis only in the presence of NAD (nicotinamide adenine dinucleotide) and that transferase II is blocked by toxin and NAD[4,5,6]. The effects of phenomycin and toxin on protein synthesis in cell-free systems from rabbit reticulocytes and rat liver have been examined in the presence and absence of NAD. The results are presented in this communication.

Diphtheria toxin (1 Lf=50 MLD in guinea pig=2 μg protein) was kindly given by Dr. I. KATO, Institute of Medical Science, University of Tokyo.

Phenomycin was observed to inhibit protein synthesis in both systems. Approximately 50 % inhibition was demonstrated at a concentration of 1 μg/ml of the antibiotic in the rat liver system, and at a little higher concentration in the rabbit reticulocyte system. No significant difference of activity was observed in the presence and absence of NAD. Diphtheria toxin inhibited protein synthesis with NAD, but the inhibition was much less without NAD. In the presence of NAD, about 50 % inhibition was demonstrated at a concentration 5 Lf/ml of toxin in the rat liver system, and at lower concentration in the rabbit reticulocyte system. In the absence of NAD, no inhibition of protein synthesis by toxin was observed in the reticulocyte system, and much less inhibition was observed in the liver system. The results are summarized in Table 1. They indicate that the mechanism by which protein synthesis is inhibited by phenomycin is different from that of diphtheria toxin. Association with NAD is essential for the latter, but not for the former.

Table 1. Effects of phenomycin and diphtheria toxin on protein synthesis in cell-free systems obtained from rabbit reticulocytes and rat liver.

Series		Relative incorporation of ^{14}C-leucine			
		Rabbit reticulocytes		Rat liver	
		no NAD	plus NAD	no NAD	plus NAD
Complete		100 (5, 280)*	100 (4, 990)*	100 (4, 430)*	100 (3, 620)*
Phenomycin	100 μg/ml	37	39	30	29
	10	39	40	38	38
	1	78	85	53	48
Diphtheria toxin	50 Lf/ml	96	13	81	47
	10	104	15	97	47
	5	101	22	97	55
	2	100	88	96	64

* cpm/mg protein.
The reaction mixture, in 0.2 ml, contained: ribosomes 0.3 mg, pH 5 fraction 0.18 mg, ATP 0.4 μmoles, GTP 0.006 μmoles, creatine phosphate 0.4 μmoles, creatine phosphokinase 10 μg, NAD 20 μg, ^{14}C-leucine 0.04 μC, Tris-HCl 2 μmoles, pH 7.8, Mg acetate 1 μmole, KCl 12 μmoles, and 2-mercaptoethanol 1.2 μmoles. It was incubated at 37°C for 40 min. The radioactivity in the hot 5 % TCA-insoluble fraction was determined in a windowless gas flow counter.

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