THE EFFECT OF PROPAMIDINE ON BACTERIAL GROWTH[1]

THE aromatic diamidines, employed in the treatment of protozoal infections,[2,3] also possess antibacterial action.[4] Propamidine (4:4'-diamidino-diphenoxy propane), used locally in the treatment of wounds, was found[5] to inhibit bacterial growth in complex media and to be uninfluenced by *p*-aminobenzoic acid. These results have been confirmed and extended.

The tests were carried out at 37.7° C., using a standard size of inoculum diluted from an actively growing culture. Ten generations were necessary to produce the degree of turbidity, measured photoelectrically, taken as positive growth. In each trial a series of drug concentrations was tested in order to determine which inhibited the rate of growth by 50 per cent. (*i.e.*, doubled the time taken by the controls to reach the chosen turbidity). Complete inhibition could be obtained by doubling or tripling this concentration.

The effect of peptone was determined with a strain of *E. coli* which grows well in medium SG of inorganic salts and glucose.[6] As shown in Table 1, adding

TABLE 1

DIAMIDINE CONCENTRATIONS INHIBITING GROWTH BY 50 PER CENT. THE FIGURES IN BRACKETS INDICATE THE NUMBER OF TESTS AVERAGED

Organism	Medium	pH	Propamidine*		Stilbamidine	
			mg per cent.		*mg per cent.*	
E. coli	SG	7.2	0.32	(2)	0.60	(1)
	SG + 1 per cent. P	7.2	0.85	(2)	4.20	(1)
	PPFG	7.7	0.85	(2)	...	
	PPFG	7.0	1.10	(3)	...	
Staph. aureus	PPFG	7.7	0.43	(3)	12.60	(2)
	PPFG	7.0	1.52	(2)	12.60	(1)

* Dr. Bernheim frequently used M/80,000 or 0.53 mg per cent. to inhibit the respiration of *E. coli*.

1 per cent. proteose peptone number 3 (Difco) approximately tripled the required amount of propamidine, whereas in parallel experiments it was found to raise the required sulfathiazole concentration by more than 500 times. Medium PPFG[6] contained 2 per cent. peptone and 0.2 per cent. glucose, added after autoclaving. Changing its pH from 7.0 to 7.7 slightly increased the drug activity. *Staphylococcus aureus* was about as sensitive to the drug as *coli;* raising the pH from 7.0 to 7.7 increased the potency of the drug 3 to 4 times. Stilbamidine was definitely less effective than propamidine. Preliminary testing in urine showed propamidine to be active at less than 4 mg per cent.

In addition, Dr. F. Bernheim and Dr. C. Brindley found the growth of the tubercle bacillus strain H37 to be inhibited about 50 per cent. by 5 mg per cent. propamidine.

The small peptone effect in the case of propamidine suggests that the *in vitro* testing of the drug will be much less difficult than has been the case with the sulfonamides. Also, this fact suggests propamidine to act primarily upon catabolic rather than anabolic systems. This is consistent with Dr. Bernheim's finding that propamidine is a potent inhibitor of cellular oxidations, particularly those involving peptone or meat extracts. As with growth, respiration is inhibited more effectively at alkaline reaction.

Methylene blue has been tested for antagonism in growth experiments. It was without effect in the case of *coli*. In *aureus*, where it is inhibitory, it synergized with propamidine. Sulfathiazole showed synergism with propamidine in *coli* grown in medium SG. In both these cases of synergism, the expected inhibition was doubled.

In *aureus* the inhibition develops gradually during the first two or three divisions (50 to 70 minutes); in *coli* the drug acts more rapidly. In *aureus* the latent period is not shortened by preliminary incubation in medium with the drug at 5° C. for 150 minutes, or 37.7° in buffer with drug. However, incubation at 37.7° C. in buffer plus *glucose* and drug about doubled the inhibition when growth was subsequently initiated by the addition of peptone. This curious result contrasts with the sulfonamides[6] and suggests that the latent period may involve activation of the drug.

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THE NATURE OF MYASTHENIA GRAVIS[1]

MYASTHENIA GRAVIS is a slowly progressive but fatal fatiguability and weakness of muscles. Walker's observation[2] that prostigmine, a choline esterase inhibitor, aided patients with myasthenia gravis, suggested that the acetylcholine metabolism was disturbed in such patients. Assuming that this concept has validity, the dominant possibilities concerning myasthenia gravis are: (a) excessive destruction of acetylcholine due to unusually large amounts of choline esterase

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2 *Proc. Roy. Soc. Med.*, 28: 759, 1935.