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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1 Aided by a grant from the Rockefeller Foundation. The drugs were kindly supplied by Merck and Company.

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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.

(demonstrated to be unlikely by Milhorat[3]); (b) defects in the ability of muscle to utilize acetylcholine (disproven by Lanari[4] and Harvey and collaborators[5,6]); and (c) defects in the synthesis of acetylcholine.

The most plausible hypothesis is that there exists a fundamental defect in the synthesis of acetylcholine in patients with myasthenia gravis. Dr. Otto Loewi suggested that such synthesis be investigated. We are immeasurably indebted to Dr. Loewi for his enthusiastic interest in the development of the problem and for his valuable advice on technique.

Method: The acetylcholine synthesis of mixtures containing standard amounts of frog brain and serum from control subjects was compared with the acetylcholine synthesis of mixtures of frog brain and of serum of patients with myasthenia gravis. Using a modified method of Quastel, Tennenbaum and Wheatley,[7] the mixtures were incubated for a standard period at a standard temperature and the acetylcholine content ascertained by means of the rectus abdominis muscle of frog (Riesser,[8] Chang and Gaddum[9]). The amount of acetylcholine synthesized during the incubation was calculated by subtracting from the content of each incubated sample the acetylcholine content of identical non-incubated samples.

Results: Mixtures containing serum and frog brain synthesized more acetylcholine than mixtures containing Ringer's solution and frog brain. Freshly prepared frog brain-serum mixtures contained 0.38 ϒ acetylcholine per 100 mg of tissue. As a result of incubation 1.45 ϒ acetylcholine was produced. In contrast the acetylcholine synthesized from the mixtures of frog brain and serum obtained from patients with advanced myasthenia gravis was approximately one third as much (0.53 ϒ). Six patients with myasthenia gravis have been compared with forty-eight control subjects.

At least some of the agents which modify acetylcholine synthesis are dialyzable.

Comment: The decrease of acetylcholine synthesis is apparently specific for myasthenia gravis, since it does not occur with other diseases presenting debility, cachexia, immobility and prostration. Also the magnitude of defect in acetylcholine synthesis is related to the severity of the myasthenia gravis.

Conclusion: The defect in acetylcholine synthesis in patients with myasthenia gravis probably explains the fatiguability and weakness of these patients.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CORTICOTROPIN OBTAINED BY ULTRAFILTRATION OF PITUITARY EXTRACTS[1]

IT is generally believed that hypophyseal corticotropin is protein in nature, as are the other hormones of the anterior pituitary, and consequently non-dialyzable. Purification has been directed towards the isolation of a protein, and dialysis has been used as a means for removing impurities of smaller molecular size. However, the stability of certain of the anterior pituitary hormones when exposed to drastic chemical procedures suggests that they may be of small molecular size.

In recent experiments it was found that dialysates of hog pituitary extracts contained a factor which increased the size of the adrenals in hypophysectomized rats. The observation was first made with a dialysate of a glacial acetic acid extract[2] of acetone-dried hog pituitary powder. The material had dialyzed for a period of two weeks against an equal volume of water, and the dialysate was used directly for injection into immature hypophysectomized male rats. In subsequent experiments extracts were ultrafiltered through Cellophane membranes (Visking sausage casing) under the pressure of a water column of approximately six feet. The ultrafiltrates were perfectly clear and almost colorless. The active material was precipitated by adding solid sodium chloride to the ultrafiltrate at a pH 4.0 until a molarity of 4.5 was reached. The subsequent addition of ammonium sulfate to a molarity of 1.5 brought about further precipitation of active material from the supernatants of the ultrafiltrates previously nearly saturated with sodium chloride. The salt precipitates were dried with ether. Another effective procedure for obtaining the activity from the original ultrafiltrates was found in the freezing-drying technique.

The amount of active substance obtained was determined by assay[2] in hypophysectomized rats. The relation of dose to response followed the parabolic assay curve which was based on the activity of hog whole pituitary powder. As the ultrafiltrates showed a

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[8] *Arch. für exper. Path. u. Pharmacol.*, 91: 342, 1921.
[9] *Jour. Physiol.*, 79: 255, 1933.

[1] Under the auspices of the Committee on Pharmacotherapy, Harvard University. Supported by the National Research Council.

[2] Details of the extraction procedure and assay method will be published elsewhere.