

levels obtained with the media of other investigators.

The above modification, with both increased concentration of egg yolk and Wilson liver powder, is being tested in a Chicago hospital which cultures some 200 stools weekly. Comparative results indicate provisionally that with this culture medium: (1) there is a greater incidence of excystation of intestinal amebæ; (2) maximal growth of amebæ taken from fresh stools is *at least* as abundant as in

other media; (3) the bacterial flora and *Blastocystis* do not grow as well in this medium.

Summary. The advantages claimed for the present culture medium for *E. histolytica* include the greater ease of preparation (and cleaning of used tubes), the prolonged life of cultures, its special suitability for studies on cellular biology, and its equal or superior adaptability for use in clinical diagnosis.

14739 P

The Anti-diamidine Activity of Sodium Nucleate.

LEAH BICHOWSKY. (Introduced by I. J. Kligler.)

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

It has been shown recently^{1,2} that aromatic diamidines which are active against protozoa *in vivo* and *in vitro* also possess antibacterial activity *in vitro* and are of value in wound therapy.

According to Fildes³ the essential action of chemotherapeutic compounds lies in their interference with the functioning of vital cell metabolites. This interference may take the form of oxidation of the metabolites, molecular combination with it, or competition for the enzyme which acts on it.

Hawking and Smiles,⁴ studying the distribution of stilbamidine in trypanosomes, found that this fluorescent substance tends to concentrate in the blepharoblast and in certain plasma granules. Adler and Tchernomoretz⁵ noted that after treatment of certain babesia infections with stilbamidine, the first signs of

degeneration occur in the nucleus of the parasites.

The present note describes an attempt to elucidate the mechanism of action of aromatic diamidines on bacteria *in vitro*.

The experiments were carried out with *B. coli* and *S. aureus* using two diamidines, stilbamidine (4:4-diamidino stilbene) and pentamidine (4:4-diamidino-diphenoxypentan). *B. coli* was grown on ammonium-phosphate, glucose, inorganic salts medium and *S. aureus* on the semi-synthetic medium of Knight.⁶ The pH was 7.3-7.4, and incubation temperature 37°C. The standard inoculum was 0.1 ml of 1:10,000 dilution of a 24-h agar slant, in 5 ml of culture medium. Inhibition was defined by absence of growth after 48 hours. The sensitivity of *S. aureus* to both diamidines is 3-4 times as great as that of *B. coli*. Stilbamidine is less potent than pentamidine against either of the two test organisms.

The addition of peptone, meat extract, or marmite to the medium lessened or abolished the effect of the diamidines, the degree depending on the amount added. Serum (10%) did not show such an effect. Substances similar in structure to the diamidines: creatin,

¹ Fuller, A. T., *Biochem. J.*, 1942, **36**, 548.

² Thrower, W. R., Valentine, F. C. O., McIndoe, A. H., Tilley, A. R., Morley, G. H., Bentley, J. P. M., Kohn, F., Hall, M. H., and Gross, C. D., *Lancet*, 1943, **1**, 133.

³ Fildes, R., *Lancet*, 1940, **1**, 955.

⁴ Hawking, F., and Smiles, J., *Ann. Trop. Med.*, 1941, **35**, 45.

⁵ Adler, S., and Tchernomoretz, J., *Ann. Trop. Med.*, 1941, **34**, 199.

⁶ Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 966.

creatinin, arginin, guanin, had no anti-diamidine activity. The following amino acids: glycine, *d,l*-methionine, *l*-tryptophan, *d,l*-phenylalanin, *d,l*-valin, *l*-leucin, *d,l*-serin, alanin, *d,l*-2-amino-n-valeric acid, *l*-oxyprolin, glutamic acid, *l*-prolin, histidin hydrochloride, lysin dihydrochloride, cystin hydrochloride, were likewise without effect.

The antibacterial activity of the diamidines was prevented when yeast sodium nucleate in suitable concentrations was added to the cultures. When sodium nucleate was added to a culture which had been maintained in a state of stasis for 24 hours by exposure to diamidine, growth developed within 24 hours.

The concentration of sodium nucleate which abolished stasis varied with the bacterial species and with the diamidine employed. Bacteriostasis induced by stilbamidine was abolished by a lower concentration of sodium nucleate than was that induced by pentamidine. A lower concentration of sodium nucleate abolished the diamidine effect on *B. coli* than that on *S. aureus*. Abolition of bacteriostasis of *B. coli* exposed to 1:25,000 stilbamidine was prevented by 0.05% sodium nucleate. Sodium nucleate of animal origin, prepared from embryo tissue, gave similar results. Separate constituents of nucleic acids (adenylic acid, adenin, guanin, uracil, xanthin, hypoxanthin) had no influence on the action of diamidines on *B. coli* and *S. aureus*.

The concentrations of nucleate required to neutralize the effect of the diamidines were rather high; however, they are of the same order as the amounts found in bacterial cells. Thus Thompson and Dubos⁷ found 2-5% of nucleic acid, dry weight, in pneumococci, and

Johnson and Coghill⁸ 0.42% in *M. tuberculosis*.

In order to ascertain whether the anti-diamidine activity of sodium nucleate preparations was due to the presence of small amounts of accessory growth substances, the effect of the following vitamins of the B-complex in concentrations of 10-100 γ per ml was tested with negative results: thiamin, riboflavin, niacin, cholin, pimelic acid, calcium-pantothenate, biotin, pyridoxin, para-aminobenzoic acid, inositol.

Prof. Adler of the Department of Parasitology examined the effect of sodium nucleate on the stasis induced by stilbamidine on *L. donovani* *in vitro*. In the serum-semisolid medium containing 1:50,000 stilbamidine, growth in the presence of 1% sodium nucleate equalled that of the controls, whereas in the absence of sodium nucleate marked growth inhibition was noted.

Trichomonas vaginalis fails to grow in Locke-goat-serum medium containing stilbamidine in a concentration of 1:300,000. In the presence of 1% sodium nucleate growth occurs even in a stilbamidine concentration of 1:5,000.

In view of these results and of the fact that in protozoa diamidines tend to concentrate primarily within bodies rich in nucleic acids, it is suggested that diamidines as basic substances combine with nucleic acids and nucleoproteins, causing a blockage of these essential metabolites.

Summary. Experiments reported above show that sodium nucleate added to the substrate counteracts the inhibiting effect of diamidines on the growth of *B. coli*, *S. aureus*, *L. donovani*, and *Tr. vaginalis*.

⁷ Thompson, R. H. S., and Dubos, R. J., *J. Biol. Chem.*, 1938, **125**, 65.

⁸ Johnson, T. B., and Coghill, R. D., *J. Am. Chem. Soc.*, 1925, **47**, 2838.