SPECIAL ARTICLES

THE EFFECT OF PROPAMIDINE ON BACTERIAL METABOLISM[1]

THE oxidative metabolism of bacteria is not affected by sulfanilamide or its derivatives in concentrations which effectively inhibit their growth. Recently it has been shown[2] that propamidine, a diamidine which is active against a number of protozoan infections, inhibits the growth of bacteria in wounds. It was therefore of interest to determine whether propamidine in concentrations which inhibit growth inhibits the oxidative metabolism of bacteria. *E. coli, Staphylococcus aureus* and *albus* were grown overnight in a medium containing 10 gms of peptone, 10 gms of glucose 3.0 gms of Armour's meat extract and 5 gms of NaCl per liter. The bacteria were centrifuged and washed with water and finally suspended in 0.05 M phosphate buffer pH 7.8. Aliquots were placed in Warburg vessels and the oxygen uptake measured in the presence of added substrates. In the absence of substrates the oxygen uptake was negligible. Nephelometric determinations before and after the experiment showed that no growth occurred during the time the bacteria were shaken in the vessels. This was true even in the presence of meat extract, the oxidation of which tends to proceed in an S-shaped curve. Table 1 illustrates an experiment with *E. coli,* which is typical also of the other bacteria tried.

This experiment shows that the oxidation of constituents of meat extract and peptone is much more sensitive to the drug than that of pyruvic acid or glucose. It is probable that the oxygen uptake in the presence of meat extract or peptone is caused by the oxidation of amino acids, for alanine and asparagine are similarly affected as shown both by the inhibition of the oxygen uptake and ammonia production. The oxidation of glucose and pyruvic acid can be inhibited effectively if the concentration of the drug is increased to M/8000. This concentration is still twenty times smaller than the 0.1 per cent. recommended for application to wounds. As indicated in Table 1, the oxidation of pyruvic acid is more sensitive to the drug than that of glucose. The figures in Table 1 also show that it takes a certain length of time before the inhibition reaches a maximum. This latent period may be attributable to the time necessary for the penetration or activation of the drug because if it is shaken with the bacteria for two hours before the addition of the substrates an immediate inhibition is obtained. At pH 6.7 the inhibition is much less effective than at pH 7.8. Thus at the end of three hours the oxidation of peptone at pH 6.7 is inhibited 29 per cent., at pH 7.8, 68 per cent. The effect of stilbamidine is similar to that of propamidine, except that higher concentrations are necessary to produce equivalent inhibitions.

The oxygen uptake of suspensions of rat kidney and liver is not inhibited by M/8000 propamidine. Addition of various substrates such as succinate, choline, xanthine, d-amino acid and tyramine shows that only the oxidation of the last is inhibited by the drug. The oxygen uptake of rat brain suspensions is, however, inhibited. This is due primarily to the fact that the oxidation of glucose is inhibited 50 per cent. by conceneration of M/30,000. It requires a concentration of M/15,000 to produce a similar effect on the oxidation of pyruvate.

TABLE 1

THE EFFECT OF M/80,000 PROPAMIDINE ON THE OXYGEN UPTAKE OF E. COLI IN THE PRESENCE OF 2.0 MG EACH OF VARIOUS SUBSTRATES, pH 7.8 37°

Time	Oxygen Uptake											
	Peptone			Meat Extract			Pyruvic Acid			Glucose		
	Control	Propamidine	Inhibition	Control	Propamidine	Inhibition	Control	Propamidine	Inhibition	Control	Propamidine	Inhibition
min.	*cmm*	*cmm*	*per cent.*	*cmm*	*cmm*	*per cent.*	*cmm*	*cmm*	*per cent.*	*cmm*	*cmm*	*per cent.*
30	16	16	0	9	9	0	28	27	0	93	98	0
60	29	22	24	22	15	32	73	66	10	191	197	0
90	46	26	44	46	24	48	127	115	10	253	269	0
150	84	32	62	141	45	68	198	178	10	322	345	0
210	121	36	70	219	80	64	222	200	10	380	397	0

SUMMARY

M/80,000 propamidine inhibits the oxidation of the nitrogeneous constituents of the medium in which the bacteria grow. A certain latent period occurs before the inhibition reaches a maximum and the drug is more effective at pH 7.8 than at pH 6.7. As is shown in the accompanying article by Dr. Kohn, this concentration is in the minimal effective range for the inhibition of growth. A latent period and a similar pH effect also are present when growth is inhibited. It is thus possible to conclude that propamidine, unlike sulfanilamide and its derivatives, directly affects the oxidative metabolism of these bacteria.

FREDERICK BERNHEIM

SCHOOL OF MEDICINE,
DUKE UNIVERSITY

[1] Dr. D. F. Robertson of Merck and Company very kindly supplied the propamidine and stilbamidine.

[2] W. R. Thrower, F. C. O. Valentine, A. H. McIndoe, A. R. Tilley, G. H. Morley, J. P. Bentley, F. Kohn, M. H. Hall and C. D. Cross, *Lancet,* 144: 133–140, 1943.