Synergistic Fungistatic Effect of Tetrazolium–Saliva Mixtures

THE presence of an antifungal factor has been reported in normal human serum and in the conjunctival mucosa[1-3]. This work was initiated to determine whether saliva possesses an identical or similar substance. Stimulated whole saliva, parotid and sub-maxillary secretions were collected from ten apparently normal individuals and tested by placing aliquots in cylinders on Pagano–Levin agar, lawn seeded with *Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*. No zones of inhibition were observed. Serum constituents (globulins) excreted in saliva are approximately one tenth of their concentration in serum and so it was decided to concentrate the salivary specimens to dryness by lyophilization. The solid constituents were then resuspended in sterile distilled water to give final concentrations 2·5–20 times greater than in saliva. Such concentrations manifested antifungal properties when tested as previously described. No inhibitory activity was discernible when Sabouraud's glucose agar, blood brain heart infusion agar or brain heart infusion agar were used, and so it seemed that the constituent in the Pagano–Levin medium which might be responsible for this activity was triphenyltetrazolium chloride, a redox indicator. The compound is reduced by *Candida tropicalis* to a red pigmented crystalline formazan whereas strains of *Candida albicans* usually fail to do so. At least five times the concentration of tetrazolium salt in Pagano–Levin medium is necessary for inhibition.

Lyophilized whole saliva or parotid or sub-maxillary secretions, concentrated twenty times, gave zones 25–30 mm in diameter, whereas specimens concentrated two and a half times produced zones of 12 mm diameter. Saliva from some individuals did not possess the antifungal factor and stringy mucinous salivas showed less activity than did non-viscid salivas. Individuals ill with upper respiratory infections showed little or no activity during the period of the acute symptoms of the disease.

The factor has been found to be filterable through glass fibre paper, 'Berkefeld', 'Selas' and 'Millipore' filters. Centrifuged specimens had fungistatic activity in the supernatant but not in the sediment. The factor is dialysable and can be concentrated by evaporation at 100° C. According to Louria and Brayton[4] sera of normal individuals contain a factor inhibitory to *Candida albicans* and *Candida stellatoidea* but not towards other *Candida* species, *Cryptococcus neoformans* or *Saccharomyces cerevisiae*. The factor these investigators have described was active after subjection to a temperature of 70° C for 1 h, dialysable, and had a molecular weight of approximately 15,000. Igel and Bolande[5] reported a serum factor which was non-dialysable and was inactivated at 70° C, but which was inhibitory towards the *Cryptococcus neoformans*. They also found a factor in saliva which inhibited *Cryptococcus neoformans*, but was distinct from the serum factor in that it was dialysable and the diffusate remained active after being subjected to 70° C for 2 h.

Our findings disagree with those of Louria and Brayton in that serum samples did not show inhibitory activity on our test medium, whereas saliva samples did. As suggested by the findings of Igel and Bolande, the factor in saliva is apparently distinct from that present in serum.

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Phosphorylation of Pyridoxine by *Escherichia coli* B

THE recent demonstration of a pyridoxine phosphate oxidase in extracts of *Escherichia coli*[1,2] and the accumulation of pyridoxine phosphate by a mutant of this organism blocked in this step[3] suggests that this oxidation is the ultimate step (*A*) in the biosynthesis of pyridoxal phosphate. If this is true, the kinase step (*B*) may be expected to have greater specificity for pyridoxine than for pyridoxal.

$$\text{common metabolite} \rightarrow\cdots\rightarrow \text{pyridoxine} \xrightarrow{B} \text{pyridoxine phosphate} \xrightarrow{A} \text{pyridoxal phosphate}$$

This communication reports the testing of this hypothesis.

Cell-free extracts of *E. coli* were prepared by ultrasonic disruption of 1 g of lyophilized cells in 25 ml. of 0·15 molar potassium phosphate at *p*H 7·0. The broken cells were centrifuged for 30 min at 2° C at 40,000*g* and the supernatant fluid was dialysed overnight against 4 l. of distilled water at 5° C. Pyridoxal phosphate was assayed by the apotryptophanase method[4]. Pyridoxine phosphate was first converted to pyridoxal phosphate by the rabbit liver oxidase method of Wada and Snell[5] and then assayed. Pyridoxine phosphate was used to standardize this reaction. The optimum concentration of magnesium sulphate for the kinase with either substrate was 10^{-4} molar. Protein was measured by the method of Lowry *et al.*[6]. Both substrates were chromatographically pure.

Table 1 shows that *E. coli* extracts can phosphorylate pyridoxine at much lower concentrations than they can phosphorylate pyridoxal. This finding and the oxidase findings are evidence in support of the foregoing biosynthetic sequence for pyridoxal phosphate.

Table 1. ACTIVITIES OF PYRIDOXINE AND PYRIDOXAL AS SUBSTRATES FOR PHOSPHORYLATION*

	mμmoles of substrate	Duration of kinase reaction (min)	Duration of oxidase reaction (min)	mμmoles product formed
(1)	None added	60	30	None detected
	5, pyridoxine	60	30	1·5
	15, pyridoxine	60	30	5·5
	25, pyridoxine	60	30	6·0
	40, pyridoxine	60	30	6·5
	50, pyridoxine	60	30	9·8
(2)	None added	5	30	0·9
	260, pyridoxine	5	30	9·0
	250, pyridoxal	5	30	1·1
(3)	None added	60	0	1·2
	250, pyridoxal	60	0	1·2
	2,500, pyridoxal	60	0	1·7

* Kinase reaction mixture contained substrate, 250 mμmoles magnesium sulphate, 1,200 mμmoles of ATP, 2 mg of protein from cell-free *E. coli* preparation in 2·5 ml. of 0·15 molar potassium phosphate, *p*H 7·0. Reaction at 37° C and stopped by subjection to 100° C for 3 min.

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Influence of Challenge Strain on Potency of Pertussis Vaccines in Mice

Eldering, Holwerda and Baker[1] have recently shown that, in mouse-protection tests with pertussis vaccines, the species-specific agglutinogen (factor 1) is more important than the type-specific agglutinogens (factors 2 and 3). This confirms my own findings[2] and those of Anderson