SODIUM PROPIONATE AND ITS DERIVATIVES AS BACTERIOSTATICS AND FUNGISTATICS

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The microbiological and pharmacological properties of sodium propionate which have been reported in previous communications,[1,2] suggested the possibility of obtaining simple derivatives which may exhibit greater activity against micro-organisms, while retaining a low order of toxicity in animals. Further *in vitro* studies have now been made of the effects of sodium propionate and certain of its derivatives on bacteria and fungi in an attempt to gain some information regarding the mechanism of the inhibitory action and to achieve enhancement of efficiency.

General Microbiology

Minimal bacteriostatic concentrations of sodium propionate for common organisms after 48 hours at *p*H 7 are shown in Table I and it is apparent that the most susceptible bacteria are largely Gram-negative,

TABLE I

Inhibitory concentrations of sodium propionate for common micro-organisms

Inhibitory concentration, per cent.	Bacteria	Fungi
0·1 to 1·5	*Pseudomonas aeruginosa* *Salmonella typhosa* *N.gonorrhœae* *B. cereus* *Serratia marcescens*	*Candida albicans* *Aspergillus niger* *Aspergillus fumigatus* *Epidermophyton interdigitale*
1·6 to 3·0	*Staphylococcus aureus* (Heatley) *Staphylococcus aureus* (Webb) *Staphylococcus albus* *Proteus vulgaris* *Streptococcus dysgalactiae* *Streptococcus agalactiae* *Streptococcus uberis* *Diplococcus pneumoniae*	— — — — — — —
3·1 to 4·5	*Staphylococcus aureus* (S. 53) *Staphylococcus aureus* (F.D.A. 209)	— —
4·6 to 6·0	*Escherichia coli* *Streptococcus pyogenes*	— —

although no generalisation is possible. The growth of pathogenic fungi appears to be inhibited by relatively low concentrations, but it has been found that certain non-pathogenic organisms of the *Penicillium* type continue to multiply in the presence of 5 per cent. or more of the compound.

The technique previously adopted[1] in studying the development of resistance has been modified by subculturing various bacteria in media

containing progressively larger concentrations of sodium propionate or sulphacetamide sodium until each group failed to grow. For convenience in interpretation, the quotient obtained by dividing the final bacteriostatic concentration by the initial bacteriostatic concentration was termed the "resistance index" and it was found that the value for sodium propionate rarely exceeded 1·5, although that for sulphacetamide sodium was invariably greater.

Mechanism of Bacteriostasis

Concentrations of sodium propionate which are capable of inhibiting growth for prolonged periods are not necessarily bactericidal and since some species of *Penicillium* and *Cladosporium* grow in media containing 6 per cent. of the compound, it would seem that the effect is not one of direct cell-toxicity. This deduction is supported by the observation that the toxicity for animal tissues appears to be very slight. Tests with a number of organisms at *p*H ranging from 8 to 5 show that bacteriostatic and fungistatic activity increases as the media are made less alkaline or more acid; greater inhibition of growth is apparent at *p*H 4, but at this value, the effect of hydrogen ion concentration alone is considerable. In acid solutions, the compound is only feebly dissociated and thus the toxic action is apparently produced by the molecule rather than the ion.

The structural similarity between propionic acid, alanine and certain other amino-acids may appear to offer a possible explanation of the mechanism on the basis of substrate competition. To test this theory, *Pseudomonas aeruginosa* was cultured on nutrient agar containing varying amounts of sodium propionate and DL α-alanine but the results, which are shown at Table II, indicate that the amino-acid does not materially affect bacteriostatic action. It has also been found that the addition of urea and histamine produces no significant change in the inhibitory concentration of sodium propionate.

TABLE II

Effects of sodium propionate and alanine on *Pseudomonas aeruginosa* (N.C.T.C. 7244)

Sodium propionate, per cent.	Alanine, per cent.	Growth after:	
		24 hours	48 hours
0	0	+	++
0·7	0	–	–
0·5	0	–	+
0·4	0	tr	++
1·0	1·0	–	–
0·5	0·5	tr	+
0·4	0·4	+	++
0·5	2·5	–	–
0	2·5	++	++
0	1·0	++	++
0	0·5	+	++

SODIUM PROPIONATE AND ITS DERIVATIVES

Since it was previously observed[1] that some antagonism may exist between sodium propionate and sulphacetamide sodium when these agents are present in equal concentration, it was decided to investigate the effects of small amounts of the sulphonamide on the activity of sodium propionate and to ascertain the influence of *p*-aminobenzoic acid. The latter substance was neutralised with sodium bicarbonate before use and *p*H of the nutrient agar was adjusted to approximately 7 in all cases. As may be seen from Table III, 0·1 per cent. or less of sulphacetamide sodium had little effect, but 1 per cent. of sodium *p*-aminobenzoate markedly increased the concentration of sodium propionate required to achieve bacteriostasis.

TABLE III

EFFECTS OF SODIUM PROPIONATE, SULPHACETAMIDE SODIUM AND SODIUM PARA-AMINOBENZOATE ON *Pseudomonas aeruginosa* (N.C.T.C. 7244)

Sodium propionate, per cent.	Sulphacetamide sodium per cent.	Sodium *p*-aminobenzoate, per cent.	Growth after:	
			24 hours	48 hours
0	0	0	+	+++
0·4	0	0	trace	++
0·75	0	0	–	–
0	0·1	0	++	+++
0·6	0·05	0	trace	++
0·6	0·1	0	–	–
0	0	1·0	+	+++
0·4	0	1·0	+	+++
0·75	0	1·0	trace	+++
1·0	0	1·0	trace	+

Low concentrations of sodium propionate appear to be oxidised quite readily by *Pseudomonas aeruginosa* with the production of carbon dioxide, but respiration is progressively reduced as the bacteriostatic level is approached. Inhibition of growth is probably associated with accumulation of sodium propionate within the cell and interference with normal carbohydrate metabolism; the dehydrogenase system may be principally affected in this way. The derivatives selected for study were designed to reduce oxidation by micro-organisms and to increase the inhibition of normal respiration, in an endeavour to obtain indications of more active groupings and, if possible, to gain further data regarding the antibacterial mechanism of sodium propionate.

BACTERIOSTATIC ACTIVITY OF DERIVATIVES

Solutions of the sodium salts of the following compounds were prepared with *p*H of approximately 7·0 and these were tested for bacteriostatic and fungistatic activity in neutral media:—α-bromopropionic acid; β-bromopropionic acid; αβ-dibromopropionic acid; α-bromopropionamide; α-hydroxy-β-phenylpropionic acid; β-phenoxypropionic acid.

The results, which are summarised in Table IV, show that simple substitution products exhibit greater activity than sodium propionate especially against *Proteus vulgaris* and *Staphylococcus aureus*. Some slight

influence may, however, be exerted on the results by the tendency of solutions of these derivatives rapidly to become acid, *p*H changes being observed after about 24 hours.

Under the conditions of testing, the bacteriostatic concentrations are generally much lower than those of sulphacetamide sodium, but these substitution products are of no therapeutic interest on account of their unstable nature. It would appear, moreover, that the bromopropionates

TABLE IV

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DERIVATIVES OF PROPIONIC ACID

Compound	Concentration, per cent.	Growth after 48 hours at 37° C.				
		Pseudomonas aeruginosa	*Proteus vulgaris*	*Staphylococcus aureus*	*Candida albicans*	*Epidermophyton interdigitale*
Sodium α-bromopropionate	0·1	+	trace	+	+	+
	0·25	–	–	–	+	–
	0·5	–	–	–	+	–
	1·0	–	–	–	–	–
Sodium β-bromopropionate	0·25		–	–	+	+
	0·5	–	–	–	+	
Sodium α, β-dibromopropionate	0·5	+			trace	
Sodium α-bromopropionamide	0·1	+		+	+	
	0·25	trace	–	+	trace	–
	0·5	–	–	–	–	–
Sodium α-hydroxy-β-phenylpropionate	0·1	+			+	
	0·25	+	–	–	+	–
	0·5	–	–	–	–	–
Sodium β-phenoxypropionate	0·1	+			+	
	0·25	–	–	–	trace	–

exert some toxic effects on animal tissues. More desirable properties may be possessed by certain derivatives of β-phenylpropionic acid, a compound which is related to substances occurring in normal metabolism, although it is less important than propionic acid in this respect. Tests with solutions of sodium β-phenylpropionate adjusted to *p*H 7 and *p*H 9 have shown that *Pseudomonas aeruginosa* (N.C.T.C. 7244) is not inhibited at 0·75 per cent. although sodium propionate at this concentration completely suppresses growth; the five organisms quoted in Table IV, moreover, continue to grow in neutral media containing 0·5 per cent. of sodium β-phenylpropionate. The latter compound is apparently metabolised by certain organisms, but it is considered that the union of halogen and possibly other radicals with carbon of the benzene nucleus is likely to overcome this effect and to enhance bacteriostatic and fungistatic activity.

INTERPRETATION OF RESULTS

Sodium propionate inhibits the growth of a considerable number of pathogenic bacteria and fungi, but it is interesting to find that species of

Penicillium and *Cladosporium* continue to multiply on media containing 6 per cent. of the compound, since propionates are frequently used as preservatives for bread and other foods, especially in America. The bacteriostatic and fungistatic effect is probably produced by the molecule, rather than the ion, and it is suggested that inhibition of growth is associated with interference with the normal carbohydrate metabolism of the micro-organisms. Streptomycin appears to interfere with the utilisation of pyruvate and although this antibiotic is a much more powerful bacteriostatic than sodium propionate, the types of bacteria susceptible to these two drugs exhibit similarities; Dubos[3] reported that low concentrations of sodium propionate inhibit growth of mammalian tubercle bacilli. One important difference, however, lies in the development of resistance to streptomycin within a short period.

The precise mechanism of the antibacterial action of sodium propionate may prove difficult to explain since activity *in vitro* does not necessarily parallel therapeutic efficiency. For example, various local infections in animals have often responded very rapidly to the compound in concentrations which represented the minimal bacteriostatic levels determined by culturing. The clinical efficiency of sodium propionate has now been demonstrated by Tucker[4] who employed this agent in the treatment of 188 animals suffering from various infections, 50 cases having previously resisted therapy with penicillin, sulphonamides and other drugs.

Sodium salts of various substitution products of propionic acid have been found to possess relatively high bacteriostatic and fungistatic activity, but certain of these substances have pharmacological and pharmaceutical disadvantages. Interesting properties may, however, be exhibited by certain derivatives of phenylpropionic acid and it is intended to investigate selected compounds in due course.

Summary and Conclusions

1. The bacteriostatic and fungistatic activity of sodium propionate against 21 micro-organisms is described briefly and it is suggested that the toxic effect is produced by the molecule rather than the ion.

2. Alanine, urea and small amounts of sulphacetamide sodium or histamine do not materially affect the bacteriostatic concentration for *Pseudomonas aeruginosa*, although *p*-aminobenzoic acid exhibits some antagonistic action. Low concentrations of sodium propionate are oxidised by this organism, but respiration is progressively reduced as the bacteriostatic level is approached, the inhibition of growth probably being associated with interference with normal carbohydrate metabolism. There is evidence that the compound is more effective *in vivo* than *in vitro*.

3. Certain substitution products possess relatively high antibacterial and antifungal activity and although none of those investigated is considered suitable for therapeutic use, the study of further derivatives is justified.

References

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