SOME ANTIBACTERIAL PROPERTIES OF SODIUM PROPIONATE

By W. W. Heseltine and L. D. Galloway

From the Technical Division Harker Stagg Limited, and The Laboratories, Welbeck Way, London.

Received May 25, 1951.

Using the agar cup-plate technique, Keeney *et al.*[1] found that a propionate ointment compared very favourably with a 5 per cent. sulphathiazole ointment against *Staphylococcus aureus* and β-hæmolytic *Streptococcus.* More recently, Theodore[2,3] has listed groups of fungi and bacteria of which growth is inhibited by sodium propionate; these organisms include, Fungi: — *Trichophyton, Epidermophyton, Microsporum, Candida, Aspergillus, Pityrosp. ovale;* Bacteria: —*Staphylococcus aureus, Staphylococcus albus, Pneumococcus, Pseudomonas æruginosa, Escherichia coli, Streptococcus.*

Our investigations are concerned with a short-term assessment of the activity of sodium propionate against common pathogens, and with *in vitro* studies of the bacteriostatic efficiency of the compound, as compared with that of sulphacetamide sodium, employing pharmaceutical preparations in clinical use. This sulphonamide was selected since, like sodium propionate, it is widely used for topical application to infected areas.

General Microbiology

Various micro-organisms were used in a short study of the activity of sodium propionate at different *p*H values, agar, broth and blood media being employed as applicable. A number of tests showed that the nature of the medium made little material difference to the results with any particular organism, and the presence of blood did not appear to affect the activity of sodium propionate, as may be seen from Table I.

TABLE I

EFFECT OF SODIUM PROPIONATE ON THE GROWTH OF *PSEUDOMONAS ÆRUGINOSA* AND *PROTEUS VULGARIS*

GROWTH AFTER 48 HOURS

Organism	Medium	Sodium Proprionate per cent.			
		0	1	2·5	5
Pseudomonas æruginosa	Blood Agar	+ +	0	0	0
,, ,, ...	Nutrient Broth	+ +	0	0	0
Proteus vulgaris	Nutrient Agar	+ +	+ +	+	0
,, ...	Blood Agar	+ +	+ +	tr	0
,, ...	Nutrient Broth	+ +	+ +	+	0

It was observed that the inhibition of fungi and bacteria increased as the *p*H passed from the alkaline to the acid side; this factor may be of

considerable importance in clinical application owing to the reaction of the skin, nasal and vaginal mucosae and possibly other tissues. Examples of the effects of *p*H are provided in Table II.

TABLE II

EFFECT OF SODIUM PROPIONATE ON THE GROWTH OF *ASPERGILLUS NIGER*, *ASPERGILLUS FUMIGATUS*, AND *CANDIDA ALBICANS* MALT AGAR CULTURES AT *p*H 7, 6 AND 5 ; GROWTH AFTER 96 HOURS

	*p*H 7			*p*H 6			*p*H 5		
Sodium Propionate per cent.	0	0·5	1	0	0·5	1	0	0·5	1
Aspergillus niger	+ +	+	+	+ +	+	tr	+ +	0	0
Aspergillus fumigatus ...	+ +	+ +	+ +	+ +	+	+	+ +	+	tr
Candida albicans	+ +	+	tr	+	0	0	+	0	0

Staph. aureus, Ps. æruginosa, E. coli and *Proteus vulgaris* cultured on nutrient agar were similarly inhibited more readily by sodium propionate at *p*H 7 than at *p*H 8. The inhibitory concentrations for certain common bacteria after 48 hours at *p*H 7 may be summarised as follows:—

Organism	*Inhibitory Concentration of Sodium Propionate.* per cent.
Ps. æruginosa	approx. 1·0
S. typhosa	
Staph. aureus (Heatley)	< 2·5
Staph. aureus (Coagulase + ve)	> 2·5
Staph. aurant. (Coagulase - ve)	
Proteus vulgaris	
Dipl. pneumoniae	
E. coli	approx. 5·0
Strep. pyogenes	> 5·0

Marked inhibition was caused by 1 per cent. or less of sodium propionate in the case of neutral cultures of *S. typhosa, Ps. æruginosa* (complete suppression at about 1 per cent.), *Staph. aureus* and *Dipl. pneumoniæ*, but rather more than 1 per cent. was required to reduce appreciably the growth of *Proteus vulgaris, E. coli* and *Strep. pyrogenes.* In these general tests, we employed only a limited range of fungi, since the fungistatic and fungicidal properties of sodium propionate have been fully investigated by Theodore[3]; Peck and his associates[4,5,6,7], Keeney[8] and other workers.

Sodium Propionate Compared with Sulphacetamide Sodium

Although bacteriostatic activity *in vitro* may not parallel therapeutic efficiency, it was considered desirable to compare the effects of similar concentrations of sodium propionate and of sulphacetamide sodium against important pathogenic bacteria. For this series of tests, the following pharmaceutical preparations were used throughout:

(a) Sodium Propionate Solution 10 per cent.; *p*H approx. 7·2.
(b) Sulphacetamide Sodium Eye Drops N.F. 10 per cent.

Group 1. COMPARISON OF ACTIVITY

Organisms—Ps. aeruginosa, Staph. aureus (Heatley strain), *Staph. aureus* (Coagulase +ve), *Staph. aurantiacus* (Coagulase −ve).

Method—Quadruple strength nutrient broth was diluted with the test solutions, and sterile distilled water if necessary, to produce the desired concentrations of the drugs. The inoculant was added in the form of 1 drop of an 18-hour broth culture and the preparation was incubated at 37°C.

Results—Growth was copious in all control cultures. The inhibitory concentrations of the two agents may be generalised by means of a simple system of brackets at 0, 1, 2·5 and 5 per cent., as in Table III.

TABLE III

Organism	Complete Suppression after 72 Hours by	
	Sodium Propionate per cent.	Sulphacetamide Sodium per cent.
Ps. aeruginosa	< 1·0	< 1
Staph. aureus (Heatley)	< 2·5	> 1
Staph. aureus (Coagulase + ve)...	> 2·5	< 1
Staph. aurantiacus (Coagulase − ve)	> 2·5	> 1

Group 2. ACCLIMATISATION EXPERIMENTS

Organism—Staph. aureus (Heatley strain).

Method—The organism was grown at 37°C. for 4 days in nutrient broth containing 0·8 per cent. of sodium propionate or of sulphacetamide sodium. It was then subcultured for 4 days in broth with 1·25 per cent. of the separate drugs, and loopfuls were subsequently used to inoculate broth containing varying concentrations of the compounds. The resultant culture was incubated for a further period of 72 hours before estimating growth.

Results—Comparison with 8-day control cultures indicated that the organism did not become appreciably resistant to sodium propionate. There was, however, some evidence of acclimatisation to sulphacetamide sodium, since the treated culture exhibited multiplication in the presence of 3 per cent. of this drug.

Group 3. TEST FOR SYNERGISM OR ANTAGONISM

Organism—Staph. aureus (Heatley strain).

Method—The method adopted was similar to that employed in Group I, but in this test for synergism or antagonism the organism was cultured in broth containing various concentrations of a mixture of sodium propionate 50 per cent. and sulphacetamide sodium 50 per cent.

Results—Growth of the staphylococcus was evident after 24 hours in the presence of 4 per cent. of the mixture (i.e., 2 per cent. of sodium pro-

pionate + 2 per cent. of sulphacetamide sodium). Comparison with controls and reference to Table III thus indicate that some antagonism may exist between these drugs.

Interpretation of Results

Although the sulphonamides have some effect on certain strains of *Trichophyton, Aspergillus niger* and species of *Actinomyces,* their antifungal activity appears to be very limited, and many organisms, including common yeasts, are resistant to them. Solutions of sulphacetamide sodium, indeed, frequently form good growth media and the ready multiplication of moulds necessitates the use of preservatives, such as benzalkonium chloride U.S.P., which is probably superior to the esters of *p*-hydroxybenzoic acid for this purpose. Since it is apparent that sulphacetamide sodium has only a limited activity against a restricted range of fungi, we decided to omit the latter organisms from the comparative tests.

Sodium propionate becomes more active against bacteria and fungi as the media are made less alkaline or more acid, so indicating an enhanced efficiency at the *p*H of the vagina, nasal secretion and skin. Although it is generally believed that the activity of sulphonamide solutions depends upon the anion and that antibacterial efficiency may, within certain limits, increase in alkaline media, it would appear that the effects of *p*H vary with the drug and also with the organism under test. Solutions containing 10 per cent. of sulphacetamide sodium have *p*H about 9, and this value may be adjusted to approximately 7·4 with 0·1 per cent. of sulphacetamide, as described in the B.P.C., or to about 6·9 with boric acid, as in the case of the N.F. and B.P.C. eye drops. Further acidification, however, results in precipitation of sulphacetamide, and it was considered impracticable to compare, by our method, the activity of sulphacetamide sodium with that of sodium propionate at the *p*H of certain tissues.

Bigger[9] has shown that boric acid and sulphathiazole are mutually antagonistic, and has suggested that it may be inadvisable to use boric acid in conjunction with sulphonamides. We have not ascertained whether sulphacetamide sodium is similarly affected, but any disadvantage induced by antagonism is possibly offset by the use of certain media which may contain substances which potentiate sulphonamides. Winkler and Julius[10] stated that a sulphanilamide-activating principle is present in the red cells of horse blood, although not in the blood of man, sheep, rabbits or mice; on the other hand, Bigger and Ware[11] found that a sulphonamide-potentiating " L substance " is present in commercial meat essences and extracts, dehydrated beef and horseflesh, commercial yeast extracts, human serum and urine and hæmolysed human and horse red blood-cells, this " L substance " being different from the principle responsible for the Harper and Cawston effect.

Our tests are intended only to provide an indication of the respective clinical values of sodium propionate and sulphacetamide sodium pre-

parations in local infections, since sodium propionate is applied topically to the eyes, ears, nose, vagina, skin and other tissues, but it is not administered systemically. In these local infections, the large numbers of organisms usually present, as well as substances such as those occurring in pus and necrotic tissue, reduce the activity of sulphonamides and, although the sensitivity of bacteria is probably increased to only a limited, variable extent by the Harper and Cawston technique, the use of lysed horse blood in culture media for tests of this kind may thus produce false results. The sulphacetamide sodium, however, may gain advantage in our investigations through light inoculation of the media and through the use of media containing 0·5 per cent. of "Lab-Lemco" and 1·0 per cent. of "Oxoid" peptone.

Summary and Conclusions

Although the species and strains of organisms used in our short study are limited, the following inferences appear to be justified:—

1. Sodium propionate inhibits growth of a wide range of bacteria and fungi responsible for local infections.
2. The activity of the drug is not markedly affected by the presence of whole horse blood.
3. The compound exhibits greater bacteriostatic and fungistatic effects in acid media than in alkaline media.
4. When tested *in vitro* in neutral media, sodium propionate appears to be rather less active than sulphacetamide sodium against staphylococci. This conclusion does not appear to be confirmed by unpublished results of clinical trials and *in vivo* investigations undertaken with the collaboration of veterinary practitioners; the variance may be due to factors such as difference in strains and the presence of sulphonamide antagonists in infected areas.
5. There is evidence that bacteria may develop resistance to sulphacetamide sodium more readily than is the case with sodium propionate, and this inference has been supported by therapeutic trial.
6. Tests with *Staphylococcus aureus* suggest that antagonism exists between sodium propionate and sulphacetamide sodium. There thus appears to be no justification for the use of a mixture of these drugs, especially since the addition of the sulphonamide would introduce the risk of sensitisation.

We desire to acknowledge the valuable assistance of Mr. S. J. Tucker.

References

1. Keeney, Ajello, Broyles and Lankford, *Johns Hopkins Hosp. Bull.*, 1944, **75,** 417.
2. Theodore, *Arch. Ophthal.*, 1949, **41,** 83.
3. Theodore, *J. Amer. med. Ass.*, 1950, **143,** 226.
4. Peck and Rosenfeld, *J. invest. Dermat.*, 1938, **1,** 237.
5. Peck, Rosenfeld, Leifer and Bierman, *Arch. Derm. Syph.*, 1939, **39,** 126.
6. Peck and Russ, *Arch. Dermat. Syph.*, 1947, **56,** 601.
7. Peck, *Chem. Products*, 1950, **13,** 176.
8. Keeney, *Johns Hopkins Hosp. Bull.*, 1943, **73,** 379.
9. Bigger, *Lancet*, 1950, **259,** 46.
10. Winkler and Julius, *J. Microbiol. Serol.*, 1942, **8,** 86.
11. Bigger and Ware, *Lancet*, 1950, **259,** 427.