STUDIES ON THE EFFECT OF PENICILLIN UPON GRAM-NEGATIVE BACTERIA. PENICILLIN-SULPHONAMIDE SYNERGY

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(With 3 Figures in the Text)

The possibility that certain Gram-negative bacteria, usually regarded as resistant to penicillin, may show a degree of sensitivity to higher concentrations of this substance has been explored by Helmholtz & Sung (1944) and Schwartzman (1946) among others, while the synergic action between a sulphonamide and penicillin, first shown by Ungar (1943), has been applied by Bigger (1946) to *Salmonella typhi*.

The following investigations attempted to determine the levels of sensitivity of a range of representative Gram-negative bacteria to penicillin and to penicillin and sulphathiazole combined. The organisms were also examined for penicillinase production.

METHODS

Media. Phosphate-buffered, nutrient broth, cleared of sulphonamide antagonizing factor by the method of Harper & Cawston (1945), was tubed in 2 c.c. amounts. Penicillin and/or sulphonamide were added from concentrated stock solutions to give final concentrations of the order shown in Table 1.

Penicillin. Crystalline sodium penicillin (Glaxo) with a potency 1650 units/mg., and a DCL* sodium salt with a potency of 685 units/mg. were used.

Inoculum. Each tube was inoculated with a standard drop of the test organism from broth cultures of various ages. The size of the inoculum was adjusted, where necessary, by making tenfold dilutions of the original culture. A suitable dilution was used for colony counts in agar to give the approximate number of organisms in the inoculum and in growing cultures.

Readings. The inhibitory effect of the penicillin and sulphonamide was estimated by visual turbidity, according to the standards shown in Table 2.

Tubes were incubated at 37° C. for 96 hr., turbidity readings being taken at 24, 48, 72 and 96 hr. After 24 hr., one-loop subcultures were made on blood agar treated with penicillinase and *p*-aminobenzoic acid (5 mg. %). Following the 48 hr. reading, penicillinase and *p*-aminobenzoic acid were added to the tubes, and further subcultures made when necessary. One-loop sterility was interpreted as partial lysis. A clear tube at the end of 96 hr. was interpreted as complete lysis of the inoculum.

p-Aminobenzoic acid was used in concentrations of approximately 5 mg. %. Ungar (1943) has shown that this substance may exert a synergistic action with penicillin against strains of *B. subtilis* and *Staphylococcus aureus*, but in our studies no synergism was shown against the Gram-negative strains tested.

Penicillinase production. The supernatant from a centrifuged, 48-hr. broth culture was added to penicillin solution, in equal volumes, to give a final concentration of 10 units/c.c. This was assayed against a standard 10-unit solution by the agar-cup technique, using the Oxford *Staphylococcus* 'H' strain as the test organism.

With this technique, the 10-unit controls gave zones with a mean value of 30·5, the standard deviation being 1·096. The majority of the organisms tested gave zones within the range 28·5–32, which did not differ from the mean value of the controls by more than twice the standard deviation. It was therefore deduced that this group of organisms did not form any anti-penicillin factors in broth. The remainder of the organisms (seven in number, as shown in Table 3) gave no zones of inhibition, and the presence of a penicillinase was assumed. When higher concentrations of penicillin were added to the supernatants (100 units/c.c.) intermediate zones sometimes occurred, indicating the possibility of quantitative variations in anti-penicillin potency, but this was not analysed further.

The method described gave results similar to those obtained using Seitz-filtered broth cultures, and was more convenient for routine use. Filtered cultures, however, were always used in testing strains of *Proteus* for penicillinase production.

Since some strains were able to inactivate penicillin in excess of the rate of inactivation of control solutions at 37° C., without producing a filterable penicillinase, the supernatant from penicillinase-broth cultures was tested similarly. No fresh penicillin was added to the supernatants, which were assayed against controls kept at 37° C. for the same period. This method was used to determine the periods for which penicillin remained active in the cultures under test.

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Table 1. *Experiment showing, as an example, the behaviour of* Salmonella paratyphi *B (type* 1*) in broth containing penicillin and sulphathiazole*

Inoculum = 200,000 organisms per c.c.

															Media
Penicillin (units/c.c.) ...	2	4	8	16	32*	2	4	8	16	32*	—	—	—	—	
Sulphathiazole (mg.%)	—	—	—	—	—	5	5	5	5	5	5	10	20	—	
Grades of turbidity:															
5 hr.	2	1	0	0	0	2	1	0	0	0	2	2	2	2	Broth
24 hr.	4	4	2	1	0	3	1	0	0	0	3	3	2	4	
48 hr.	.	.	4	3	2	3	1	1	1	1	3	3	2	.	
72 hr.	.	.	.	4	4	4	3	3	3	3	4	4	4	.	Broth with *p*-aminobenzoic acid and penicillinase
96 hr.	.	.	.	.	.	.	4	4	4	4	.	.	.	.	
Subcultures at 24 hr.	*c*	*c*	*c*	*d*	—	*c*	*d*	*d*	*d*	—	*c*	*c*	*c*	*c*	Agar with same

c = confluent growth on subculture.
d = discrete colonies on subculture.
— = one-loop sterility.

* Penicillin concentrations up to 128 units/c.c. were used with less sensitive organisms, e.g. *Bact. coli.*

Table 2

Degree of turbidity	Appearance	Approximate number of coliform organisms (millions/c.c.)
0	Clear	No growth
1	Slight opalescence or granular deposit	15 million in controls. Involution forms in cultures under suppression
2	Turbid or granular	200
3	Turbid	700
4	Full 24 hr. turbidity of control culture	More than 1000

Table 3

				Bacteriostatic levels		
					Penicillin (unit/c.mm.)*	
Organism	Behaviour in penicillin	No. of strains	Penicillinase production	Sulphathiazole (mg.%)	Alone	With sulphathiazole
S. typhi	Sensitive	2	—	20	8	2
S. paratyphi B	Sensitive	8	—	20	16–20	2·5–6
S. typhi-murium	Sensitive	1	—	20	20	10
Proteus	Sensitive	4	—	20–50	10–20	5
	Resistant	1	—	—	—	—
Proteus morgani	Resistant	3	—	20–50	—	—
Bact. coli	Sensitive	11	—	10–50	30–100	10–30
	Resistant	1	1	—	—	—
	Variable	1	1	20	100	20
Paracolon group	Sensitive	2	—	10–20	100	20–30
	Resistant	4	3	10–50	—	—
Bact. aerogenes	Sensitive	1	—	—	60	—
	Resistant	5	2	—	—	—
Bact. friedlanderi	Sensitive	2	—		See text	
	Resistant	2	—	—	—	—

* These refer to the concentrations required to maintain cultures at grade 1 turbidity for 24 hr. or more.

RESULTS

An example of the ordinary method of testing is shown in Table 1 and the findings for the various groups of Gram-negative bacteria are shown in Table 3.

General findings

Penicillin. Sensitivity varied considerably. *S. typhi* was inhibited by 8 units; typical strains of *Proteus vulgaris* and *S. Paratyphi* B required 10–20 units for the same grade of inhibition; a few strains of *Bact. coli* were inhibited by 30–50 units; while *Bact. aerogenes*, *Paracolon* bacilli, *Bact. friedlanderi* and other strains of *Bact. coli* either showed no demonstrable sensitivity or required 100–120 units. The concentrations shown in the table refer to 24 hr. turbidity readings. When earlier readings were made, suppression of growth was found to occur at lower concentrations during the first 5–9 hr., at which stage the control cultures had already entered the logarithmic phase and produced turbidity.

The coliform organisms which were insensitive to 120 units of penicillin could be placed in two main groups:

(*a*) Those which produced a filterable penicillinase in growing broth cultures and completely destroyed penicillin in concentrations of 100–120 units/c.c. in the course of their growth. This group included strains of *Bact. coli*, *Paracolon* and *aerogenes*.

(*b*) Those which grew at a normal rate in high concentrations of penicillin but produced no filterable penicillinase. The penicillin in the growing cultures was inactivated more slowly and sometimes incompletely after 48 hr. *Proteus morgani*, *Bact. friedlanderi* and some strains of *Bact. coli*, *Paracolon* and *aerogenes* behaved in this fashion.

Suppression of growth by penicillin was rarely maintained for more than 48 hr., except in those considerably higher concentrations which sometimes produced lysis of the relatively sensitive members of the series. Even where the bacteriostatic effect was marked, the addition of penicillinase at any stage enabled the culture to proceed rapidly to full growth. *Sulphonamides* were used in concentrations of 5–50 mg. %. Growth was usually suppressed to some extent by concentrations greater than 10 mg. %, but little or no effect was obtained by 5–10 mg. %, which is comparable to the blood level attained by 4-hourly dosage. Sulphathiazole appeared to be most active, sulphanilamide least active. Sulphadiazine and sulphapyridine were intermediate.

Though not preventing the early multiplication of the organisms, sulphonamides were found to exert a prolonged action so that a degree of suppression which was reached after 24 hr. was maintained and sometimes increased thereafter, until the addition of *p*-aminobenzoic acid allowed the culture to proceed to full growth.

Synergic action. When penicillin and sulphathiazole, sulphadiazine or sulphapyridine were both present, considerably lower concentrations of each agent sufficed to inhibit growth (Table 3 and Fig. 1). The best results were obtained with sulphathiazole, and in some instances the combined action permitted of a fourfold reduction in the concentration of penicillin required for bacteriostasis and

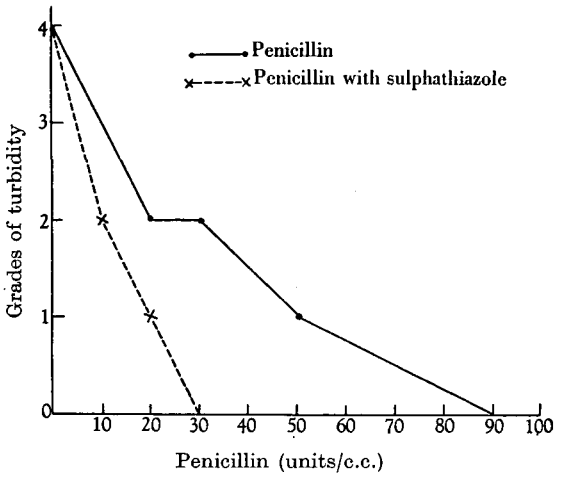

Fig. 1. Grades of turbidity attained in 24 hr. by a strain of *Bact. coli* growing in broth in the presence of penicillin alone and penicillin with sulphathiazole (5 mg. %).

lengthened the effective period of suppression. The addition of *p*-aminobenzoic acid and penicillinase enabled cultures which had not undergone lysis to proceed rapidly to full growth.

In spite of the enhancement of the bacteriostatic effect, the combined action of the two substances did not usually produce a significant lowering in the level of penicillin required for lysis. Essentially, it seemed that the combined action was a bacteriostatic one.

Size of the inoculum. The standard inoculum was 100,000–500,000 organisms per c.c., and the results shown in Table 1 refer to this. When the inoculum was below 10,000, bacteriostasis was produced by slightly lower levels of penicillin and, conversely, inocula of more than 1,000,000 required higher concentrations.

Age of culture. Experiments were performed with inocula from 48- and 96-hr. cultures. Growth was inhibited by penicillin alone and by penicillin-sulphathiazole at the same concentrations as younger organisms, but lysis by the appropriate higher concentrations of penicillin was less readily obtained and was seldom complete. In this sense,

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older cultures appeared to be more refractory to the action of penicillin.

Differences in penicillin. When regard was paid to the strength in units and not to the bulk of the preparations, the crystalline penicillin and the impure product gave comparable bacteriostatic levels, alone and in the presence of sulphathiazole. It seemed that lysis occurred more constantly and at lower concentrations with the pure product, but this was not studied in detail.

Morphological changes. It was noted that cultures under suppression by penicillin or penicillin-sulphonamide appeared granular. Where the suppression was only partial (grade 2) the medium had a light, granular turbidity, but where suppression was fairly complete (grade 1) the medium was almost clear, except for a slight granular deposit. When such cultures were centrifuged and carbol-fuchsin-stained films compared with those made from the control cultures, it was found that the granular masses were composed largely of involution forms of the bacteria. In the subinhibitory concentrations, these appeared as long, evenly staining filaments, but in higher concentrations they showed abnormal pleomorphism and irregular staining. The most bizarre forms were obtained from cultures under the combined action of penicillin and sulphathiazole, and included clumps of beady filaments, spore-like objects and feebly stained threads. When subcultured on blood agar, containing *p*-aminobenzoic acid and penicillinase, these granular deposits yielded larger colonies, which tended to remain discrete, but were composed of organisms of normal morphology.

Sulphonamides alone, in the concentrations tested, produced no abnormal morphological changes in the bacterial cells.

Behaviour of individual bacteria

Salmonella typhi. The behaviour of this organism was studied in some detail, in parallel with clinical studies which it is hoped to describe elsewhere. The strains used belonged to phage type E1 and, in general, the results confirmed the findings of Bigger (1946).

Penicillin inhibited growth for a few hours in concentrations of 2–4 units/c.c.; 24 hr. bacteriostasis was produced by 8 units and complete lysis of an inoculum of 10,000–500,000 organisms by 16–20 units. In the presence of sulphathiazole (5 mg. %) the bacteriostatic effect produced by 2 units of penicillin was maintained for 48 hr. In the same concentrations (5 mg. %) sulphapyridine and sulphadiazine required 4 and 6 units of penicillin respectively. Sulphanilamide showed no synergism with penicillin. These inhibitory effects were also obtained in media containing 50 % human serum and plasma.

Salmonella paratyphi B. This organism was slightly less sensitive to penicillin than *S. typhi*, 16–20 units being required for bacteriostasis and 20–50 for partial lysis. A marked synergic effect was obtained with sulphathiazole, when 2·5–6 units of penicillin produced bacteriostasis for 24–48 hr. The synergic effect was maximal with sulphathiazole but negative with sulphanilamide. The behaviour of various phage types is shown in Table 4.

Salmonella typhi-murium was rather less sensitive to both agents, but showed similar behaviour.

Proteus. The effect of penicillin upon strains of *Proteus vulgaris* was described by the author in a previous paper (1945), where it was shown that concentrations of 5–10 units/c.c. exerted an inhibitory effect in various liquid media during the first few hours of growth. A bacteriostatic effect lasting 24 hr. was produced by 10–20 units, while

Table 4. *Salmonella paratyphi* B

Phage type	No. of strains	Bacteriostatic levels of penicillin (units/c.c.) Alone	With sulphathiazole
1	3	16	4
2 (3B)	1	16	2·5
3A	1	20	6
3B	2	16	4
Not typed	1	16	4

a variable degree of lysis resulted with higher concentrations. These findings were confirmed with fresh strains, and sulphathiazole was found to have an inhibitory effect in concentrations of 20–50 mg. %. A well-marked synergic effect was obtained, whereby the presence of sulphathiazole (5 mg. %) enabled 5 units of penicillin to produce bacteriostasis lasting 25–48 hr.

Four strains behaved in this way, two being isolated from urine (cystitis), one from a case of puerperal sepsis and one from the blood culture of a case with a pyonephrosis and septicaemia. These strains possessed cultural and biochemical properties typical of *P. vulgaris*, swarming readily and producing acid and gas in glucose and xylose. The septicaemia strain was serologically related to *x* (*K*) strains, as tested by the reciprocal absorption of immune serum and patient's serum, which agglutinated the organism to a titre of 1 in 12,800.

Two other types of *Proteus* behaved differently:

(*a*) *P. morgani* was completely resistant to penicillin in concentrations up to 128 units/c.c. Sulphathiazole caused some inhibition in concentrations of 20–50 mg. %, but no synergic effect was exhibited. No penicillinase was produced. The three strains tested were isolated from faeces, urine and an empyema.

(*b*) An atypical, mannitol-fermenting strain was isolated from a case of otitis externa. This organism was insensitive to penicillin and sulphathiazole alike, and there was no synergic effect. No penicillinase was produced.

The biochemical reactions of this strain were highly unusual. Acid and gas were formed in glucose, saccharose, mannitol and maltose. Cultures swarmed readily and produced H_2S and indole.

Bact. coli. Thirteen strains isolated from faeces, urine and various lesions were examined.

Eleven strains showed a sensitivity to penicillin within the range 30–100 units/c.c., while all the strains were inhibited to some extent by sulphathiazole in concentrations of 20–50 mg. %. The presence of sulphathiazole (5 mg. %) enabled penicillin to inhibit the sensitive strains at concentrations of 10–30 units/c.c. Figure 1 shows the behaviour of a representative urinary strain.

Two penicillinase-producing strains were identified. One of these was completely insensitive to penicillin, but the other could be inhibited when a small inoculum (<100,000 organisms/c.c.) was added to broth containing 100 units of penicillin/c.c., though not when the penicillin was added to give the same final concentration in a 5-hr. culture. This strain was inhibited by sulphathiazole, acting alone, in a concentration of 20 mg. % and a marked synergic effect was obtained with penicillin (20 units/c.c.) and sulphathiazole (5 mg. %). The behaviour of this strain suggested, therefore, that penicillin could exert some action on the bacterial cells where the concentration of penicillinase was low. This action was maintained by the presence of a subinhibitory concentration of sulphathiazole, just as with non-penicillinase strains.

No correlation was found between the ordinary biochemical properties of these strains and their response to penicillin, nor between strains isolated from faeces, urine or pus. This applied also to the other coliform organisms described below.

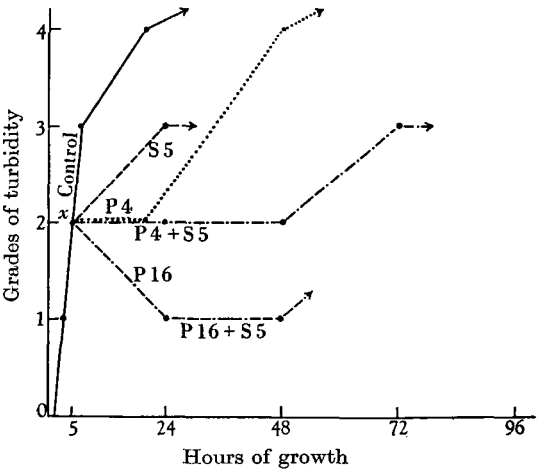
Paracolon bacilli. Six strains were tested, isolated from faeces, urine and pus. Two strains showed sensitivity to penicillin in the high concentration of 100 units. Sulphathiazole showed some activity in the range 10–50 mg. %, and in a concentration of 5 mg. % its presence enabled penicillin to inhibit the two sensitive strains at 20 units/c.c. No synergism was obtained with sulphanilamide. Three of the remaining four strains produced a highly active penicillinase.

Bact. aerogenes. Six strains were tested. Four were isolated from faeces and two from urine, one being also present in a blood culture. One (faecal) strain was inhibited by 60 units of penicillin. Sulphathiazole showed no bacteriostatic activity, and no synergic effect could be demonstrated with any of the strains.

Bact. friedlanderi. Four strains, isolated from sputum, were tested. Penicillin inhibited one strain at a concentration of 60 units/c.c., while sulphathiazole showed bacteriostatic activity against this strain at 20 mg. %. A weak synergic effect was obtained with the combination of 20 units penicillin with 5 mg. sulphathiazole.

Of the remaining three strains, one was inhibited by 128 units of penicillin while the other two were insensitive to penicillin. Sulphathiazole showed no activity against these three strains, and no synergic effect could be demonstrated. No penicillinase was produced.

The nature of the synergic effect. An impression of this was gained by adding crystalline penicillin



P 4 = Penicillin (approximate final concentration 4 units/c.c.).

S 5 = Sulphathiazole (approximate final concentration 5 mg. %).

Both substances were added at point *x*, after 5 hr. normal growth.

Fig. 2. Addition of penicillin and sulphathiazole to a growing culture of *S. typhi*.

and sulphathiazole, separately and together, to cultures in various stages of growth and, conversely, by adding penicillinase and *p*-aminobenzoic acid to cultures under various degrees of suppression. *S. typhi* and two strains of *Bact. coli* were used as the test organisms.

The results obtained with *S. typhi* are shown schematically in Figs. 2 and 3. Similar results were obtained with *Bact. coli* when the necessary higher concentrations of penicillin were used.

Fig. 2 shows the behaviour of cultures to which penicillin, sulphathiazole or both were added after 5 hr. growth (turbidity grade 2). Sulphathiazole did not prevent further division but gave a slight reduction in the final turbidity. Penicillin

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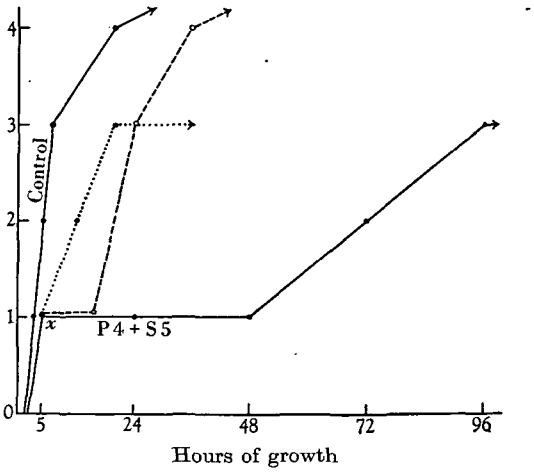
(4 units/c.c.) produced a brief bacteriostatic effect, but thereafter the culture proceeded to full growth. The addition of sulphathiazole along with or up to 5 hr. after the penicillin maintained this bacteriostatic effect for 24–48 hr., and the culture failed to grow to full turbidity. In a concentration of 16 units/c.c., penicillin caused a reduction in turbidity until 24 hr., after which the culture grew more or less normally. When sulphathiazole was present, this reduction was maintained for 48 hr. and full turbidity was not attained at 96 hr.

Fig. 3 illustrates the effect of penicillinase and *p*-aminobenzoic acid upon 5-hr. cultures under suppression by penicillin and sulphathiazole acting



Grades of turbidity:

..... Penicillinase (or S 5 alone).

o------o *p*-Aminobenzoic acid (or P 4 alone).

x = Point at which *p*-aminobenzoic acid or penicillinase were added to cultures under suppression by P 4 + S 5.

Fig. 3. Addition of penicillinase and *p*-aminobenzoic acid to a culture under suppression by penicillin and sulphathiazole.

together. Penicillinase liberated the culture to grade 3 turbidity within 24 hr. (giving precisely the same result as was obtained when sulphathiazole alone was added to a control culture after 5 hr. growth). When *p*-aminobenzoic acid was added, the culture remained at grade 1 for a few hours, and then grew rapidly to full turbidity (i.e. showing much the same behaviour as a 5-hr. control culture to which penicillin had been added to give a concentration of 4 units/c.c. The more rapid escape of the culture to which *p*-aminobenzoic acid had been added, as shown in Fig. 3, was probably due to the fact that penicillin was present from the beginning of the experiment.)

When penicillinase and *p*-aminobenzoic acid were added simultaneously, the cultures grew rapidly to full turbidity. When neither substance was added, the cultures remained at grade 1 for 48 hr. and grew slowly to grade 3 (72–96 hr.). At 24 hr., inhibition was released more readily by *p*-aminobenzoic acid than by penicillinase.

By assaying the supernatants from test cultures, as described above, it was found that penicillin became inactivated in the presence of sulphathiazole at the same rate as in ordinary broth cultures; with *Bact. coli*, this inactivation was complete at the end of 24 hr., though when sulphathiazole was present growth remained suppressed until 48 hr. This confirmed the idea that the maintenance of the bacteriostatic effect was a function of the sulphonamide.

Further experiments were made, using different concentrations of penicillin and sulphathiazole, and testing all the penicillin-sensitive organisms shown in Table 2. It was found that the synergic effect could only be exhibited when the concentration of penicillin was such that, acting alone, it produced an inhibitory effect during the first few hours of growth. When this concentration of penicillin was present, no advantage was obtained by raising the sulphathiazole concentration to an inhibitory level (20 mg. %); and when the concentration of penicillin was not in itself inhibitory a rise in the sulphathiazole concentration did not expedite the synergic effect. These findings suggest that relatively low concentrations of penicillin can exert a bacteriostatic effect upon certain Gram-negative bacteria within 5 hr.; this action is short-lived, but it renders the bacteria amenable to the action of sulphathiazole which maintains the same degree of bacteriostasis for 24–48 hr. Thereafter (48–96 hr.) the inhibitory action is comparable to that of sulphathiazole acting alone on normal bacteria. Lysis of the bacteria by penicillin, at higher concentrations, can proceed in the presence of sulphathiazole, but is not enhanced. Nevertheless, where lysis is incomplete, some advantage is obtained from the presence of the sulphathiazole by its prolonged bacteriostatic action upon the surviving organisms.

DISCUSSION

When applied to Gram-negative bacteria, the term 'sensitivity to penicillin' must be interpreted with reservations. It covers the range, in this series, of 8–128 units/c.c., while certain members of the group include strains which exhibit no sensitivity to 128 units. On the other hand, some of the more important pathogens, including *S. typhi*, *S. paratyphi* B, *Proteus* and certain strains of *Bact. coli*, are inhibited by concentrations which might be attainable and effective therapeutically.

The nature of the resistance offered by the penicillin-insensitive strains is by no means wholly answered by penicillinase production. There is a

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suggestion in these studies that penicillin is inactivated more rapidly in cultures of the less sensitive organisms, but this is not always associated with the production of a penicillinase, and some organisms may show resistance without inactivating the penicillin.

In the treatment of mixed infections, the action of penicillin on the more sensitive organisms is obviously governed to some extent by the rate of its inactivation by resistant Gram-negative organisms; if the Gram-negatives, in their measure, are sensitive, then a more complete effect might be obtained by using higher concentrations where this is practicable.

Several workers have already investigated the effect of penicillin upon Gram-negative bacteria. Helmholtz & Sung (1944) showed that coliforms could be inhibited in urine by 15 units/c.c., while Thomas & Levine (1945) showed that certain intestinal bacilli were inhibited by 5–50 units, and that involution forms appeared in subinhibitory concentrations. Previous studies by the present author (Stewart, 1945) showed that some strains of *Proteus* could be inhibited in broth and body fluids by 5–25 units/c.c. Schwartzman (1946) reported that *Bact. coli* and Salmonellas were more sensitive in a basal medium devoid of amino-acids.

The conception of a synergic action upon certain organisms between sulphonamides and penicillin is a familiar one, and has been demonstrated by Ungar (1943) and Bigger (1944). Hobby & Dawson (1944) showed that the synergic action was essentially a bacteriostatic one, and that in fact the bactericidal rate of penicillin was decreased in the presence of sulphonamide. This observation was confirmed by Garrod (1944), who found that the presence of a bacteriostatic concentration of sulphathiazole materially reduced the rate of lysis of staphylococci by penicillin.

Synergic action by the two substances upon Gram-negative bacteria has been investigated by Bigger (1946) using *S. typhi*, and Klein & Kalter (1946) using a strain of *Shigella paradysenteriae* which, they claimed, gave results representative of those obtained with organisms showing such diverse behaviour as *S. paratyphi*, *S. typhi* and *Bact. coli*. These authors contended that the synergic effect *in vitro* was obtained only when both agents were present in concentrations which were already inhibitory.

In our studies, it has been shown that concentrations of sulphathiazole (5 mg. %) which are not bacteriostatic *per se* during the first 24 hr. of growth may nevertheless act synergistically with penicillin. The very essence of the synergic effect may be in this fact, for there is a considerable amount of evidence to show that penicillin acts best on dividing organisms which would not appear if the sulphonamide concentration were completely bacteriostatic. Whether penicillin acts *only* on dividing organisms is doubtful, since cells from older cultures are not necessarily resistant. Such cells may in some instances be inhibited by the same concentration and are equally amenable to the synergic action. The high proportion of involution forms which appear in cultures under penicillin suppression and the persistence of such forms after the addition of sulphonamide suggests that penicillin may render the organisms more susceptible to the action of sulphonamide. The latter substance, acting alone, cannot produce these changes in the bacterial cells, nor can it prevent division in the first 24 hr. of growth. It is also possible, as Klein & Kalter suggest, that penicillin by its lytic action reduces the number of cells to limits within which lower concentrations of sulphonamide become effective, though the low concentrations of penicillin which can be used under synergic conditions do not seem to be exclusively bacteriolytic in their effect, and it is likely that the other effect, described above, is also concerned. In the first 10–12 hr. of growth, sulphonamide seems to play a comparatively quiescent role, but between 12 and 48 hr. it becomes actively bacteriostatic. It is not clear if there is any further chemical affinity between the two agents, though the superiority of sulphathiazole and the absence of a synergic effect with sulphanilamide in the strains tested suggests that this is possible.

SUMMARY

The effect of penicillin alone, and with sulphonamide upon various Gram-negative bacteria, has been investigated.

S. typhi, *S. paratyphi* B and strains of *Proteus vulgaris* were inhibited by penicillin in concentrations of 8–20 units/c.c. Certain coliforms were inhibited by 30–128 units.

Organisms which were inhibited to some extent by penicillin and sulphonamide acting separately were inhibited under experimental conditions at lower concentrations when both agents acted together. The mechanism of this reaction has been discussed.

A proportion of the insensitive coliform strains were identified as penicillinase producers.

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