THE COMBINED EFFECT OF PENICILLIN AND OF SULPHONAMIDES IN INFECTIONS WITH GRAM-NEGATIVE ORGANISMS. PARTS III AND IV

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PART III.

THE COMBINED EFFECT ON *SALMONELLA ENTERITIDIS*, *SHIGELLA FLEXNERI* AND *PROTEUS VULGARIS*

Similar experiments were done to study the effect of the combined action of penicillin and of the sulphonamides on these Gram-negative organisms. The experimental procedure was the same as that used with *Bact. coli* and *Salm. typhi*, and the results were equally similar[12].

In vitro, the results recorded in Tables VII, VIII and IX show that the inhibitory concentration of penicillin was reduced by 1/4th to 1/8th when the sulphonamide compounds were present in the culture medium as well; the concentration of the sulphonamide, however, was by itself non-inhibitory or only slightly bacteriostatic.

TABLE VII

THE COMBINED EFFECT *IN VITRO* OF PENICILLIN AND OF SOME SULPHONAMIDES ON THE GROWTH OF *SALM. ENTERITIDIS*

Sulphonamide 0·1 mg./ml.	Penicillin concentration in units/ml.							
	20	10	5	2·5	1·25	0·6	0·3	0
———	0	4	4	4	4	4	4	4
Sulphathiazole	0	0	0	1	3	3	3	3
Sulphapyrazine	0	0	0	2	3	3	3	3
Sulphadiazine	0	0	0	4	4	4	4	4

TABLE VIII

THE COMBINED EFFECT *IN VITRO* OF PENICILLIN AND OF SOME SULPHONAMIDES ON THE GROWTH OF *SHIG. FLEXNERI*

Sulphonamide 0·05 mg./ml.	Penicillin concentration in units/ml.							
	50	25	12·5	6·25	3·125	1·56	0·78	0
———	0	0	2	4	4	4	4	4
Sulphadiazine	0	0	0	0	0	2	2	2
Sulphapyrazine	0	0	0	0	0	1	2	2
Sulphathiazole	0	0	0	0	2	4	4	4

In vivo, there was a marked prolongation in the average survival period of mice treated with penicillin and sulphonamide combined as compared to that of animals treated by either chemotherapeutic substance alone. Table X shows this effect in mice infected with *Salm. enteritidis*.

TABLE IX

THE COMBINED EFFECT *IN VITRO* OF PENICILLIN AND OF SOME SULPHONAMIDES ON *PROTEUS VULGARIS*

Sulphonamide 0·2 mg./ml.	Penicillin concentration in units/ml.						
	100	50	25	12·5	6·25	3·125	0
————	0	0	4	4	4	4	4
Sulphadiazine	0	0	0	0	0	3	3
Sulphapyrazine	0	0	0	0	0	3	4
Sulphathiazole	0	0	0	0	0	3	4

TABLE X

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDES IN *SALM. ENTERITIDIS* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
—— ——	Sulphathiazole 1 mg.	6	1·2
2000 units	Sulphathiazole 1 mg.	6	6·0
—— ——	Sulphapyrazine 0·2 mg.	12	1·7
2000 units	Sulphapyrazine 0·2 mg.	12	4·9
—— ——	Sulphadiazine 0·2 mg.	12	4·2
2000 units	Sulphadiazine 0·2 mg.	12	6·4
—— ——	Sulphadiazine 0·1 mg.	6	3·0
2000 units	Sulphadiazine 0·1 mg.	6	4·7
2000 units		12	2·0
Untreated controls		18	0·4

2,000 units of penicillin caused an average survival period of 2 days; when this dose was combined with 1 mg. of sulphathiazole, the average survival period was markedly increased, to 6 days, 5 times as much as the survival period when the same dose of sulphathiazole was used alone. With sulphapyrazine, the average survival period was increased nearly 3 times more than that when 0·2 mg. of sulphapyrazine was given alone. When sulphadiazine was used, however, a less marked advantage was noticed from combining it with penicillin, probably because sulphadiazine was already very effective in a small dose against this infection when used alone.

Table XI shows that mice infected with *Shig. flexneri* and treated with the combined therapy enjoyed a survival time 2·6 and 4·4 times more than those treated with sulphadiazine or with sulphapyrazine alone respectively.

TABLE XI

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDES IN *SHIG. FLEXNERI* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
—— ——	Sulphadiazine 0·2 mg.	11	2·3
2000 units	Sulphadiazine 0·2 mg.	6	6·0
—— ——	Sulphapyrazine 0·2 mg.	6	1·3
2000 units	Sulphapyrazine 0·2 mg.	6	5·7
2000 units		17	0·8
Untreated controls		17	0·4

Table XII shows once more the advantage of the combined therapy, this time against *Proteus* infection in mice.

TABLE XII

THE COMBINED EFFECT OF PENICILLIN AND SULPHONAMIDS IN *PROTEUS* INFECTION IN MICE.

Treatment		Number of mice used	Average survival period days
Penicillin	Sulphonamide		
—— ——	Sulphadiazine 0·2 mg.	18	3·0
1000 units	Sulphadiazine 0·2 mg.	12	5·0
—— ——	Sulphapyrazine 0·5 mg.	6	3·0
1000 units	Sulphapyrazine 0·5 mg.	6	6·0
—— ——	Sulphathiazole 0·5 mg.	12	1·7
1000 units	Sulphathiazole 0·5 mg.	12	4·7
1000 units		18	1·6
Untreated controls		18	0·4

PART IV.

DISCUSSION AND CONCLUSIONS

1. *The nature of the combined action.* The combined effect of penicillin and of some sulphonamides on organisms known to be very sensitive to penicillin has been investigated by many workers[1-9]. Although they

agree that there is an advantage in such a combination, their conclusions differ in the interpretation of the nature of the combined action. Two terms were used by these authors; some described the combined effect as additive, while others used the term synergic. According to Gaddum[12], synergism means that two drugs which have the same effect are helping one another in their action; in this case the same effect which is produced by a certain concentration of each, can be obtained if fractions of the effective doses of either compound are used together when the sum of these fractions is less than two. If the sum of these fractions which give the same effect equals one, their effect is said to be additive; if on the other hand, the effect is produced by fractions of the two concentrations the sum of which is less than one, the drugs are said to potentiate the action of each other.

This terminology is now applied to the results of the combined action of penicillin and of the sulphonamides obtained in the *in vitro* experiments in the present investigation. It is seen that complete inhibition of the growth which was obtained by a certain concentration of penicillin or of the sulphonamides when used separately, was obtained when both substances were used together each in a concentration less than half the original one; e.g., if the inhibitory concentrations of penicillin and of the sulphonamides were 50 units /ml. and 1 mg./ml. respectively, the same effect was produced when 1/4th of the penicillin concentration (12·5 units /ml.) and 1/10th of the sulphonamide concentration (0·1 mg./ml.) were used together. This is, therefore, some evidence that this is a potentiation effect.

In vivo, the synergism between penicillin and the various sulphonamides has been also demonstrated; the results of the experiments show that this synergistic action is also of a potentiation character. A significant correlation between the doses of the drugs used and the resulting effect has been found, and the regression lines were calculated. In Part I of this paper[13] Figure 1 shows that in mice infected with *Bact. coli,* an average survival period of 5 days is obtained with 0·4 mg. of sulphapyrazine; this is 1/4th of the sulphapyrazine dose used in combination with 2,000 units of penicillin to produce the same effect (see Table III). 2,000 units of penicillin when used alone is definitely less than one half the effective dose (see Figure 2). When 0.5 mg. of sulphathiazole was used with 2,000 units of penicillin, the average survival period was found to be 5·7 days (see Table III); if sulphathiazole is to be used alone, 2·5 mg. is required to produce the same effect (see Figure 3); i.e., 5 times the dose used in combined therapy.

With *Salm. typhi,* the result of the synergistic action between penicillin and the sulphonamides[13] is recorded in Table VI. From Figure 4 it is seen that the same effect which was produced by 1 mg. of sulphadiazine when combined with 2,000 units of penicillin, can be obtained with sulphadiazine alone with 6·5 mg. are used. Also, Figure 5 shows that 8·9 mg. of sulphapyrazine are required to produce an average survival period of 6·1 days; this same effect however, was produced when 2 mg. of sulphapyrazine were used together with 2,000 units of penicillin. This dose of penicillin is less than one-half the effective dose (Fig. 6).

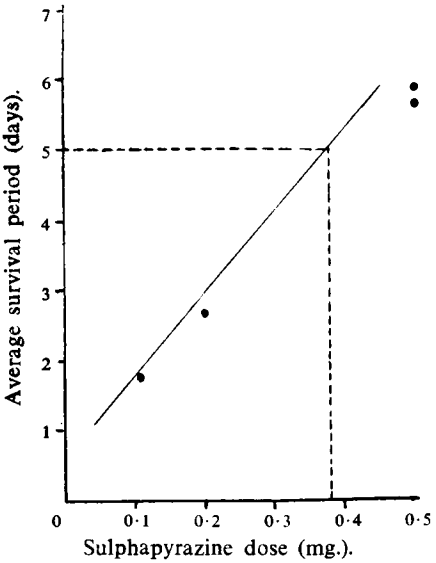

FIG. 1.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of sulphapyrazine used in treatment.

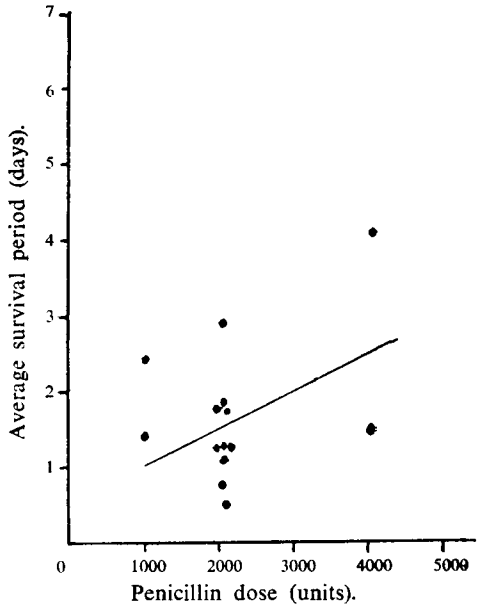

FIG. 2.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of penicillin used in treatment.

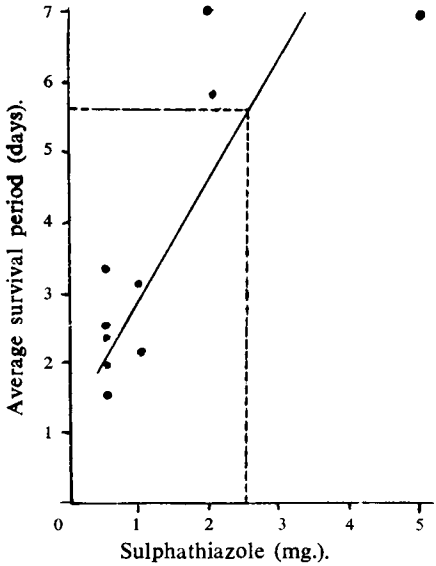

FIG. 3.—The average survival period per mouse infected with *Bact. coli* in relation to the dose of sulphathiazole used in treatment.

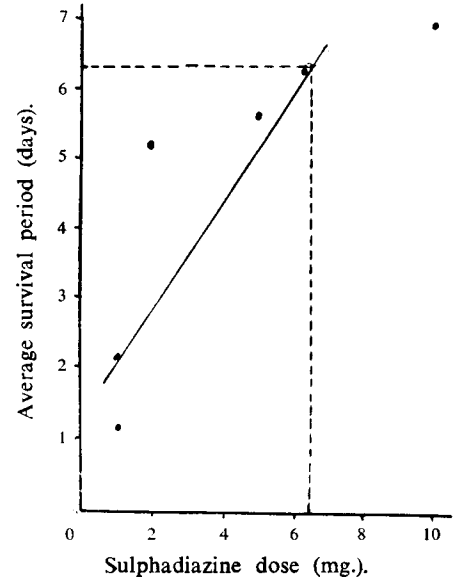

FIG. 4.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of sulphadiazine used in treatment.

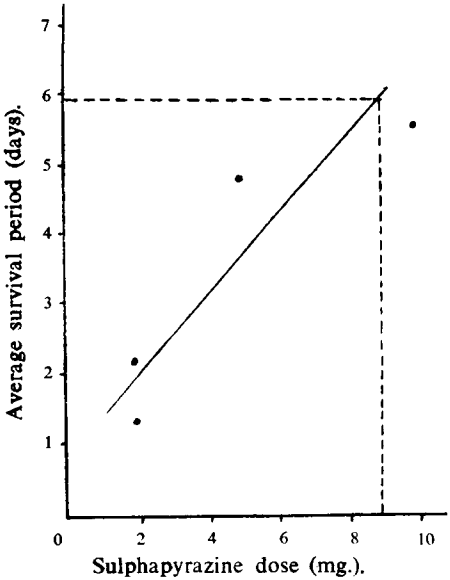

FIG. 5.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of sulphapyrazine used in treatment.

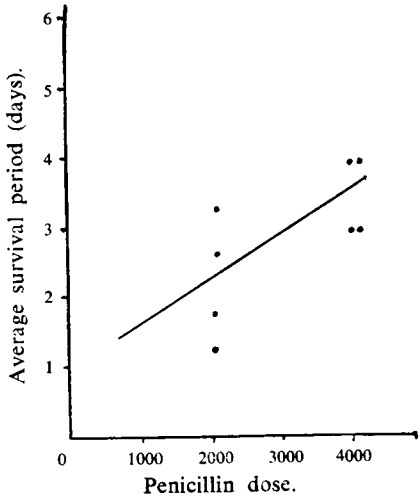

FIG. 6.—The average survival period per mouse infected with *Salm. typhi* in relation to the dose of penicillin used in treatment.

(2) *The mechanism of the combined action.* Klein and Kalter[7] found that the use of penicillin with sulphathiazole, or sulphadiazine or sulphapyrazine resulted in an increased antibacterial activity against staphylococci and streptococci *in vitro;* their explanation was that penicillin sharply reduces the total number of micro-organisms and thus permits the sulphonamides to be more active on the remaining small number of cells as these compounds are only partially bacteriostatic in the presence of large numbers of bacteria. This, however, does not explain why the synergism also occurs if the penicillin concentration was not bactericidal by itself.

Hobby and Dawson[6] explained the synergism observed between penicillin and sulphadiazine *in vitro* by suggesting that " the fact that sulphadiazine appears to increase the activity of penicillin against one strain resistant to penicillin alone suggests the possibility that penicillin may alter the bacterial cell so as to increase its sensitivity to sulphadiazine."

The work of Gardner[14,15] and of Fleming *et al.*[16] indicated that morphological changes occur in the Gram-negative bacilli when subjected to penicillin concentration inadequate for complete inhibition. The *in vitro* antibacterial activity, in the present investigation, was judged by the degree of turbidity in the medium caused by the growth of the organism; if the bacteria still grow fully in spite of the presence of a certain concentration of penicillin, it does not mean, therefore, that these bacteria were not at all affected by the antibiotic. It is suggested here that, as a non-inhibitory penicillin concentration affects the bacterial cells, although it does not kill them, this effect of the penicillin together with the partial

bacteriostatic action of the sulphonamide, may bring about complete inhibition of the growth; the two chemotherapeutic compounds thus co-operating in their action on the organism.

It is known that, although penicillin and the sulphonamides both produce inhibition of growth, they differ in the mechanism by which this end is achieved. The view mostly accepted is that penicillin is a bacterial agent[17,18], and that its acts on bacteria in the stage of active division[14,15,19,20,21]. Florey and Florey[22], however, state that penicillin is bacteriostatic and not bactericidal in its action, in concentrations likely to be produced therapeutically. According to Todd[23], the mechanism by which the organisms are destroyed is that they are first killed and then autolysed by autolysins. Chain and Duthie[5], however, stated that penicillin may abolish oxygen uptake by young organisms before cell division occurs. The sulphonamides, on the other hand, exert their bacteriostatic action by substrate competition[24,25], it is suggested that the sulphonamide molecule, being similar to that of paraminobenzoic acid, can replace it and thus deprives the bacterial cells of one of the essential metabolites. The two chemotherapeutic substances, administered at the same time, will, therefore, attack the bacterial cells from two different angles and thus interfere with their activity in a more efficient way.

(3) *Advantages of the combined therapy.* The following are the main advantages of the combined use of penicillin and of the sulphonamides observed in this study.

(a) The ultimate result obtained when the two chemotherapeutic substances were used together was an increase in the average survival period as compared to that resulting from the use of each substance alone in the same doses. It may be argued that the same therapeutic response may be obtained by increasing the dose of the sulphonamide compound, without using penicillin. However, it is definitely an advantage to use a small dose of the sulphonamide compound with penicillin than to use a much bigger dose of the sulphonamide alone, as this latter may prove to be too toxic to the host.

(b) In some conditions, the sulphonamides when used in ordinary therapeutic doses will not give a satisfactory therapeutic response, neither will penicillin even when used in very large doses. With penicillin, the difficulty is not with the size of the dose as, theoretically, this has no upper limit, but it is essential to maintain an adequate concentration in the blood and tissues during treatment. The presence of the sulphonamides in ordinary concentrations in the blood enables such a penicillin concentration as could be maintained there to exert an efficient antibacterial action, although it would exert no effect when present alone.

(c) In many instances, infection is not caused by one organism, but by a mixture of organisms which may vary widely in their sensitivity to the different chemotherapeutic substances. The use of one substance only, e.g., penicillin, may not be sufficient to eradicate the infection, and the combined therapy may be of great advantage in such cases.

(4) *Practical application of the combined therapy.* The value of the combined therapy has already been recognised in the treatment of infec-

tions caused by organisms which are more sensitive to penicillin. Dowling *et al.*[26] compared the mortality rate in two groups of patients suffering from pnuemococcal pneumonia; the first group was treated with sulphadiazine alone and the second treated with sulphadiazine and penicillin. Out of 94 patients in each group, the mortality was 9·6 per cent. in the first and 4·3 per cent. in the second. Waring *et al.*[27] treated 13 patients with pneumococcal meningitis with sulphadiazine and penicillin with one fatality only (7·7 per cent.). The authors compared these results with those of others before sulphonamide or penicillin therapy were introduced; mortality was 100 per cent. in one series of 29 patients before the introduction of sulphonamide therapy in 1936, this was reduced to 70 per cent. with sulphonamide treatment. Smith *et al.*[28] treated two groups of similar cases, 29 patients in each group; the first had penicillin only with 7 deaths and the second group was treated with penicillin and sulphadiazine with 2 deaths only, one of them was stated to be moribund on admission and the other died from an unrelated condition, fat embolism. Card *et al.*[29] observed that sulphathiazole and penicillin appear to enhance the effect of each other against *N. gonorrhœae* and that their combined used was a safe, rapid, efficient and economical method of treating gonorrheal urethritis.

Also with the more resistant organisms, the combined therapy has been applied and gave better results than single treatment. Levy and McKrill[30] compared the mortality rates in three groups of patients suffering from subacute bacterial endocarditis (due to *Streptococcus viridans*), in the first group treated with sulphadiazine alone, the mortality was 96 per cent. in the second treated with penicillin alone 40 per cent., but in the third when a combination of both substances was used, the mortality was only 28·6 per cent.

The results obtained in this experimental work suggest that a similar improvement in the prognosis of the diseases caused by the Gram negative organisms may be anticipated if they are treated with penicillin and with the sulphonamides together. The size and frequency of the doses to be used will depend on the particular infection in question. The organisms tested in this investigation cause a variety of local and systematic infections in the body which have been hitherto resistant to all forms of therapy.

Bacterium coli is a normal inhabitant of the intestines, but if it invades other systems of the body it causes various inflammatory conditions. The commonest sites affected by *Bact. coli* are the urinary tract, the gall bladder and the peritoneal cavity. Less commonly, *Bact. coli* is found in local suppurations, e.g., empyema cavities, in these there is no difficulty in maintaining the required penicillin concentration by local application. The urinary tract also is an easy situation where penicillin can reach in a high concentration; 40 units/ml. of urine is reached during treatment with 100,000 units daily and this is a very moderate dose[31]. This concentration may prove adequate to kill *Bact. coli* in the urinary tract, if not, the combination with the sulphonamides most probably will.

Salm. typhi causes a grave disease in man, the combined therapy with

penicillin and sulphathiazole has already been used with successful results[32,33,34].

Lastly, the combined therapy suggested may still prove its value in the resistant *Proteus* infection. *Proteus* is not considered as highly pathogenic, it may be found as a saprophyte in the nasal cavities and intestines. Pathologically, it is found in urinary tract infections in 6 to 13 per cent. of cases[35,36]. The fact that penicillin is found in high concentrations in the urine has already been referred to and it may prove useful in treating such cases. It has been found[37] that the average concentration required to inhibit the growth of strains of *Proteus vulgaris* obtained from infected urines was 8 units /ml. Wound infection with these organisms is occasionally met with and this is one of the conditions in which the local combined use of penicillin and of the sulphonamides should be tried. Less commonly *Proteus* septicæmia occurs, the primary infection may be in the urinary tract[38], or in local septic collection[39,40], in this condition effective treatment is more than ever needed, and the combined therapy may prove to be the answer.

Urinary tract inflammations are due, in many instances, to a mixed infection, the advantage of combined therapy in such conditions has already been referred to.

It is planned to apply this combined therapy to infections caused by other "resistant" organisms, e.g. *Streptococcus viridans,* the causative agent in the majority of cases of subacute bacterial endocarditis. Treatment of this condition with penicillin, although it has very considerably improved the prognosis in this fatal disease, gives a recovery rate of only 60 per cent[41,42]. This figure may be improved if combined therapy is instituted.

This synergistic action between penicillin and the sulphonamides is not only confined to these two drugs; it was shown, by many workers, that synergism also occurs between other pairs of chemo-therapeutic substances. Kolmer[9] demonstrated such an effect between penicillin and streptomycin against pneumococcal and anthrax infections in mice. Also, when penicillin was known to affect *Treponema pallidum,* synergism was found between penicillin and arsenic in treating experimental syphilis in rabbits[9,43]. Perhaps one of the most interesting results of the combination of two chemotherapeutic substances is its application to treatment of tuberculosis infection. Smith *et al.*[44,45], found that promin and other sulphone compounds when used alone were relatively ineffective in the treatment of experimental tuberculosis; better results were obtained when they were combined with streptomycin; the result of the combined therapy was better than the sum of the effects of the individual compounds. Callomen *et al.*[46] also showed that a much better therapeutic effect resulted by treating experimental tuberculosis in guinea-pigs with streptomycin and diasone together than by treatment with either substance alone. Brownlee and Kennedy[47] also showed that a combination of streptomycin and of various sulphone compounds produced a better effect than either agent alone.

Recently the present writers[48] demonstrated this synergism between

chloromycetin and sulphadiazine against *Salm. typhi.* infection in mice. With the rapid advance of the science of chemotherapy, the number of infections beyond control are progressively diminishing. Experimental results obtained *in vitro* or in animal experiments with a new chemotherapeutic substance are not always confirmed when practical application to human disease is attempted, nevertheless there seems to be a possibility that combined therapy with more than one therapeutic agent may be effective in conditions where one agent alone has not been successful, and it is likely that this combined therapy will be most successful when chemotherapeutic agents are employed which have a different point of attack on the infecting agent.

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