CCXXX. THE USE OF COMPOUNDS RELATED TO *p*-AMINOBENZENESULPHONAMIDE IN THE TREATMENT OF CERTAIN INFECTIONS IN MICE

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It is now well established that *p*-aminobenzenesulphonamide has some effect on septicaemias in mice other than those caused by the haemolytic *Streptococcus* [Buttle *et al.* 1936; Proom, 1937; Buttle *et al.* 1937 etc.], and further that various related compounds exert chemotherapeutic action in certain mouse infections. However, in no case has it been shown that the *in vivo* effects run parallel with the *in vitro* results, and the mode of action is still very obscure.

The object of the present investigation has been to compare the chemotherapeutic actions of a number of selected compounds on mice infected with various pathogenic bacteria. The organisms chosen were the *Streptococcus*, the *Staphylococcus*, the *Pneumococcus* and *Bacillus aertrycke*. At the same time experiments were carried out to determine what action, if any, the compounds exerted on the growth of these bacteria *in vitro* in order to ascertain whether the action *in vitro* could in any way be correlated with the chemotherapeutic activity as exhibited in animal experiments.

In vivo experiments

A. *Infection of animals.* Using the mouse as the experimental animal approximately 100 M.L.D. of the infecting organism were injected intraperitoneally. The inoculum was obtained from an 18-hr. slope culture which was emulsified and made up to the required dilution with either sterile saline or 5% mucin. Some organisms were not sufficiently virulent to kill mice when suspended in saline, and so a method involving the use of mucin similar to that of Miller & Castles [1936] was used to diminish the minimum fatal dose of these bacteria. 1 ml. of the bacterial dilution was injected intraperitoneally into mice of 20–25 g. weight.

B. *Chemotherapeutic treatment.* The various compounds under investigation were first tested for their toxicity when administered to normal mice orally through a feeding tube. Whenever possible the compound was brought into solution in a suitable solvent, but otherwise it was given in the form of a suspension in gum acacia. The doses given to the infected mice varied according to the toxicity but in each case the drug was fed immediately after the injection of organisms and at intervals thereafter. The frequency of feeding varied according to the reaction of the animal to the organism and to the drug.

The non-toxic dose employed for the majority of compounds was 10 mg. administered by mouth twice daily for 2–3 days.

Results of animal experiments. The results obtained from experiments with a series of compounds are shown in Table I. In the case of *p*-aminobenzenesulphonamide, with the *Streptococcus* as the infecting organism, the usual clear-cut

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Table I. In vivo experiments

100 m.l.p. of infecting organisms injected intraperitoneally into mice of 20–25 g.
10 mg. therapeutic agent fed immediately, after 5 hr. and twice daily for 2–3 days.

Compound	Formula	Oral toxicity for 20 g. mouse mg.	Solvent	Therapeutic activity			
				<i>Strepto-</i> <i>coccus</i>	<i>Pneumo-</i> <i>coccus</i>	<i>Staphylo-</i> <i>coccus</i>	<i>Bacillus</i> <i>aertrycke</i>
<i>p</i> -Aminobenzenesulphonamide		80	Water	+++	+ to ±	±	±
<i>p</i> -Hydroxybenzenesulphonamide		50–60	Water	± to 0	±	±	0
3-Nitro-4-hydroxybenzenesulphonamide		>100	Sodium bi-carbonate	++	±	0	0
3-Amino-4-hydroxybenzenesulphonamide		80	Suspension in gum acacia	±	±	±	0
<i>p</i> -Aminobenzenesulphonamide coupled with <i>p</i> -hydroxybenzenesulphonamide		20	Water	±	±	0	0
<i>p</i> -Aminobenzenesulphonamide coupled with 8-hydroxyquinoline		20	Suspension in gum acacia	± to 0	0	±	0
<i>p</i> -Aminobenzenesulphonamide coupled with 8-hydroxyquinolinemethosulphate		60	Water	± to 0	0	±	0
4:4'-Dinitrodiphenylsulphide		>100	Suspension in gum acacia	0	±	0	0
4:4'-Diaminodiphenylsulphide		About 60	Lactic acid	±	±	0	0
4:4'-Diacetyldiaminodiphenylsulphide		>100	Suspension in gum acacia	±	±	+ to ±	0

+++ 100% recovery, ++ 75% recovery, + 25–30% recovery, ± death delayed, 0 no effect.

evidence of chemotherapeutic activity was obtained, 100% of the animals infected recovering completely within 3-4 days. With the other compounds the best result was obtained with 3-nitro-4-hydroxybenzenesulphonamide when, in a series of haemolytic streptococcal experiments, 75% of the treated animals survived indefinitely. This compound also showed slight action against the pneumococcal infection of mice but there was no effect on the *Staphylococcus* or on *Bacillus aertrycke*. As the toxicity of this compound is less than that of *p*-aminobenzenesulphonamide its use as a therapeutic agent is worthy of consideration.

Of the other results the most important is the antistaphylococcal action of diacetyldiaminodiphenylsulphide.

In vitro experiments

Technique. When possible, solutions of the compounds were prepared; otherwise fine suspensions were employed. In every case the pH was adjusted to 7.6. Dilutions were made in broth or serum broth according to the organism to be tested. These broth tubes were inoculated with 0.1 ml. of a thick bacterial suspension obtained by emulsifying an 18-hr. slope culture of the organism, and incubated at 37° for 18 hr. In order to determine both the bacteriostatic and the bactericidal activities of the compound, subcultures from the broth tubes were made on blood-agar plates after 3, 6 and 18 hr. incubation, and from the growth readings of these plates the activity of the drug was ascertained. The results obtained with the series of compounds used in the *in vivo* experiments is shown in Table II.

Table II. In vitro experiments

Each papain broth tube was inoculated with 0.1 ml. of a thick suspension of organisms, pH 7.6, rH 19-20. Subcultures were made after 3, 6 and 18 hr., and from these readings the bacteriostatic and bactericidal activities were estimated.

Compound	Bactericidal and bacteriostatic activities			
	<i>Streptococcus</i>	<i>Pneumococcus</i>	<i>Staphylococcus</i>	<i>B. aertrycke</i>
<i>p</i> -Aminobenzenesulphonamide	+++	+++	0	± to 0
<i>p</i> -Hydroxybenzenesulphonamide	+++	++++	0	0
3-Nitro-4-hydroxybenzenesulphonamide	++++	+++	0	0
3-Amino-4-hydroxybenzenesulphonamide	++	+++	0	0
<i>p</i> -Aminobenzenesulphonamide coupled with <i>p</i> -hydroxybenzenesulphonamide	++	+	± to 0	0
<i>p</i> -Aminobenzenesulphonamide coupled with 8-hydroxyquinoline	++ to +	++++	+	±
<i>p</i> -Aminobenzenesulphonamide coupled with 8-hydroxyquinolinemethosulphate	++ to +	++++	+	±
4:4'-Dinitrodiphenylsulphide	++	++	±	0
4:4'-Diaminodiphenylsulphide	++ to +	++	±	0
4:4'-Diacetyldiaminodiphenylsulphide	++ to +	++	±	+ to ±
+++ Bacteriostatic at 1 in 10,000, bactericidal at 1 in 1000.				
++ + „ 1 in 10,000, „ 1 in 100.				
+ + „ 1 in 1000, „ 1 in 10.				
+ „ 1 in 100.				
± „ 1 in 10.				
0 No effect.				

Results of in vitro experiments. The bactericidal action of these compounds on the *Pneumococcus* is greater than is to be expected from the results of animal experiments. Though this is partly to be explained by the low viability of the *Pneumococcus* as compared with other organisms, the difference between the

antipneumococcal activities of *p*-hydroxybenzenesulphonamide and the quinoline derivatives in the test tube and in the living animal requires further investigation. Although many compounds, as is shown in Table II, are bactericidal to the *Pneumococcus*, yet in no case has it been possible to cure mice with a pneumococcal septicaemia.

The results with the *Streptococcus* in one or two cases show a slight parallelism with those obtained in animal experiments, but with the majority of compounds the antiseptic value shows little correlation with the curative power.

Apart from causing a slight delay in the growth rate in a few experiments, the compounds give almost uniformly negative results with the *Staphylococcus* and *Bacillus aertrycke*.

DISCUSSION AND CONCLUSIONS

Perhaps the most interesting result of the work here reported is the relatively high antistreptococcal activity of 3-nitro-4-hydroxybenzenesulphonamide. All the sulphur-containing compounds previously found to exercise marked chemotherapeutic activity against septicaemias caused by the haemolytic *Streptococcus* have an amino group or a group which can be easily converted into the amino group, e.g. the nitro group, either free or substituted in the *p*-position in the benzene ring. The activity of the new compound indicates that this rule is not general and suggests that it is important to prepare and test other derivatives possessing the 3-nitro-4-hydroxybenzene grouping. It will be noted that 3-nitro-4-hydroxybenzenesulphonamide is definitely less toxic than sulphanilamide. This compound was also active on pneumococcal septicaemias in mice but in this case its efficacy was definitely inferior to that of *p*-aminobenzenesulphonamide.

p-Hydroxybenzenesulphonamide, as well as certain disubstituted benzenesulphonamides, have been tested by Tréfouel *et al.* [1937] in respect of their antistreptococcal activity in mice, in every case with practically negative results. Our results with the first of these compounds are in agreement with their findings; of the disubstituted derivatives used by Tréfouel *et al.* none were available to us.

The antistaphylococcal activity of 4:4'-diacetyldiaminodiphenylsulphide is of some interest. Though the activity of this compound against the *Staphylococcus* is not very great, under favourable conditions it does delay death by about 4 days, and this indicates that antistaphylococcal action is not limited to certain sulphonamide derivatives but belongs also to other classes of sulphur-containing compounds.

The main conclusion to be drawn from a comparison of the experiments carried out on infected mice and those performed *in vitro* is a negative one. There is no obvious connexion, as far as our results show, between antibacterial actions under these two different conditions. Thus it would appear that the effect of the compound on the bacteria which is of importance for its chemotherapeutic action is not one of general toxicity, i.e. a simple inhibition of growth or metabolism, but is rather a highly specific and characteristic one. The view that this group of active sulphur-containing compounds exercise their chemotherapeutic action through some special damaging effect on the resistance of the organisms to the antibacterial action of the blood and the body tissues has been advocated by many investigators, and has recently received support from the observations of Whitby [1938]. He has shown that the remarkable chemotherapeutic action of 2-*p*-aminobenzenesulphonamidopyridine on mice infected with pneumococci is associated with the loss by the bacteria of their capsule which makes the organisms much more susceptible to phagocytosis and other similar processes. The balance of the evidence seems to suggest that some similar though perhaps

more subtle change is effected in organisms such as the *Streptococcus* by compounds of the sulphonamide group and other active sulphur-containing derivatives.

SUMMARY

1. Ten compounds have been tested in respect of their chemotherapeutic actions on mice infected with haemolytic streptococci, pneumococci, staphylococci or *Bacillus aertrycke*. The antibacterial actions *in vitro* of these compounds have also been examined.

2. 3-Nitro-4-hydroxybenzenesulphonamide has a marked curative effect on streptococcal septicaemias in mice. The low toxicity of this compound suggests that it should be investigated on a wider scale.

3. Diacetyldiaminodiphenylsulphide delays the death of mice infected with staphylococci.

4. Under the conditions of experimentation, no correlation could be established between the action of these compounds *in vitro* and their chemotherapeutic activity on infected mice. This result is in accordance with the view that the important action of these compounds on the organisms is not one of gross toxicity, but that it involves a damaging of the defences which normally protect the organisms from the bactericidal processes of the body.

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