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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.

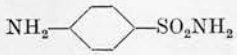
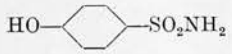
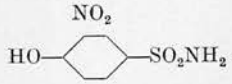
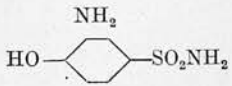
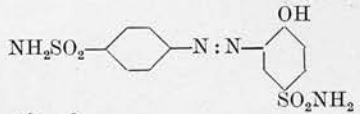
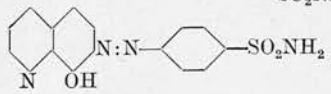
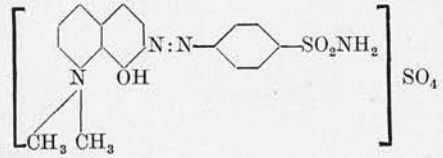
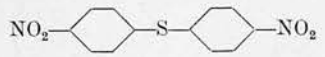
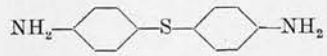
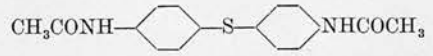
B. *Chemotherapeutic treatment.* The various compounds under investigation were first tested for their toxicity when administered to normal mice orally through a feeding tube. Whenever possible the compound was brought into solution in a suitable solvent, but otherwise it was given in the form of a suspension 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

Table I. In vivo *experiments*

100 M.L.D. 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			
				Streptococcus	*Pneumococcus*	*Staphylococcus*	*Bacillus aertrycke*
p-Aminobenzenesulphonamide		80	Water	+ + +	+ to ±	±	±
p-Hydroxybenzenesulphonamide		50–60	Water	± to 0	±	±	0
3-Nitro-4-hydroxybenzenesulphonamide		>100	Sodium bicarbonate	+ +	±	0	0
3-Amino-4-hydroxybenzenesulphonamide		80	Suspension in gum acacia	±	±	±	0
p-Aminobenzenesulphonamide coupled with *p*-hydroxybenzenesulphonamide		20	Water	±	±	0	0
p-Aminobenzenesulphonamide coupled with 8-hydroxyquinoline		20	Suspension in gum acacia	± to 0	0	±	0
p-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 *p*H 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, *p*H 7·6 *r*H 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: *Streptococcus*	*Pneumococcus*	*Staphylococcus*	*B. aertrycke*
p-Aminobenzenesulphonamide	+ + +	+ + +	0	± to 0
p-Hydroxybenzenesulphonamide	+ + +	+ + + +	0	0
3-Nitro-4-hydroxybenzenesulphonamide	+ + + +	+ + +	0	0
3-Amino-4-hydroxybenzenesulphonamide	+ +	+ + +	0	0
p-Aminobenzenesulphonamide coupled with *p*-hydroxybenzenesulphonamide	+ +	+	± to 0	0
p-Aminobenzenesulphonamide coupled with 8-hydroxyquinoline	+ + to +	+ + + +	+	±
p-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.

I wish to express my thanks to Dr W. O. Kermack for his help and encouragement during the course of this work, and for supplying me with the compounds tested.

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THE CHEMOTHERAPY OF TYPHOID

AND SOME OTHER NON-STREPTOCOCCAL INFECTIONS IN MICE

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THE CHEMOTHERAPY OF TYPHOID AND SOME OTHER NON-STREPTOCOCCAL INFECTIONS IN MICE

FOLLOWING the work of Domagk (1935) on Prontosil, *p*-aminobenzenesulphonamide was shown to be effective in the treatment of mice against intraperitoneal infection by streptococci (Tréfouël, J. and Mme., Nitti and Bovet 1936; Buttle, Gray, and Stephenson 1936; Colebrook and Kenny 1936) and meningococci (Buttle et al. 1936; Proom 1937).

In this paper we describe the results obtained in treating mice infected with various other organisms—viz., *Bacterium typhosum*, *Bact. paratyphosum* B, *Bact. aertrycke*, pneumococcus, Friedländer's bacillus, and pasteurella. The degree of protection is also compared with that previously reported from the Wellcome Physiological Research Laboratories for the hæmolytic streptococcus and the meningoccus.

TECHNIQUE

Infection of Mice.—A number of strains of each organism were obtained, and the most virulent selected for this investigation. All the organisms were grown for 18 hours in the medium described by O'Meara and Brown (1936); the cultures were diluted in broth and 0·5 or 1·0 c.cm. amounts injected intraperitoneally into mice of 25 to 30 grammes in weight. Dilutions of some of the strains were also made in 5 per cent. mucin, using a method similar to that of Miller and Castles (1936) in their work on the titration of antimeningococcus serum. The mucin produced a considerable diminution in the minimal fatal dose, an observation which has already been made by Miller (1935) for the meningococcus, and by Rake (1935) for *Bact. typhosum*. The virulence of the strains tended to decrease when they were kept on artificial media, but was restored to some extent by mouse passage.

Treatment with the Sulphonamide.—The drug was given to the mice by mouth in suspension with gum acacia using a 1 c.cm. syringe with a blunted needle. In preliminary experiments on dosage a single injection of 100 mg. was tolerated by mice of 25 to 30 g., but produced a temporary paralysis with incoördinate movements of the limbs which lasted four to five hours. Doses of 40 mg. and 25 mg. were therefore used for the present investigation and produced no symptoms.

The general scheme of treatment was to give one group of mice a single injection of the drug, and a second group injections twice daily, commencing treatment in each case immediately after infection. A third group received their first treatment five hours after infection, and thereafter two doses a day as for the second group.

BACT. TYPHOSUM

Three strains were used of approximately equal virulence—viz., "Rawlings" (rejuvenated), "Allahabad," and "Mrs. S.," all of which were kindly supplied by Major J. S. Boyd, R.A.M.C. Experiments have been made, with and without mucin, a typical protocol (strain "Rawlings") being shown in Table I.

In the first section of the experiment, no mucin was used to enhance virulence. Of 20 treated mice (2 groups of 10 animals infected with 50 million organisms and treated immediately) 15 were alive twelve days after the injection of 100 times the number of organisms (500 thousand) required to kill all 10 control mice. In the second section, in which mucin was used as a diluent for the culture, protection was obtained against a slightly larger number of fatal doses; a group of 10 treated mice, injected with 10 thousand times the number of organisms required to kill 4 of 10 controls, all survived for twelve days. (In another experiment treated mice were kept for one month, and no deaths occurred after the twelfth day.)

A single dose of 25 mg. of the sulphonamide was almost as effective as doses of 25 mg. repeated twice daily for 6 days. When treatment was delayed for five hours after infection, the result was not as good as with immediate treatment, although some protection was still demonstrable.

In these experiments we were able to isolate *Bact. typhosum* from the heart blood, spleen, and, less often, the kidney of some apparently healthy mice one week after infection and treatment. After one month a positive culture was obtained from the spleen of one of twelve mice examined, and serum tests sometimes showed the presence of agglutinins. We also found that treated mice which survived seemed to possess slight active immunity to a second infecting dose one to four weeks later. (This phenomenon has not been observed in experiments on the treatment of hæmolytic streptococcal infections in mice.)

BACT. PARATYPHOSUM

Bact. paratyphosum A strains were not used since those available were of relatively low virulence.

We tested two recently isolated, highly virulent *paratyphosum* B strains which Prof. Hedley Wright kindly sent us. It was unnecessary to use mucin as

TABLE I

Treatment of Mice Infected with Bact. typhosum ("*Rawlings*"—*rejuvenated*)

Treatment with the sulphonamide.	Approx. number of organisms in infecting dose.*	Number of mice (out of groups of 10) dying on each day after infection.				Mice surviving 12 days.
		1st	2nd	3rd	6th	
ORGANISMS INJECTED WITHOUT MUCIN						
None.	50,000	..	..	..	..	10
	500,000	5	5	..	..	0
	5,000,000	10	..	..	..	0
25 mg. twice daily.	5,000,000	..	..	..	..	10
	50,000,000	1	1	..	..	8
	500,000,000	10	..	..	..	0
Single dose (25 mg.).	5,000,000	..	2	..	..	8
	50,000,000	1	2	..	..	7
25 mg. twice daily (first dose delayed 5 hours).	5,000,000	2	..	1	..	7
	50,000,000	7	1	..	2	0
ORGANISMS INJECTED IN SUSPENSION WITH MUCIN†						
None.	50	3	1	..	..	6
	5,000	7	2	1	..	0
	500,000	10	..	..	..	0
25 mg. twice daily.	5,000	..	..	..	..	10
	500,000	..	..	..	..	10
	50,000,000	6	3	1	..	0

*As judged by opacity method, checked by serial dilutions in Wright's broth and colony counts on Wright's agar plates.

† Ten mice injected with mucin alone, and 10 mice injected with mucin and treated with the sulphonamide, all survived.

500 organisms killed 60, and sometimes 100, per cent. of mice within 48 hours of infection. Protection was obtained against 100 to 1000 fatal doses, when treatment with the drug was either a single dose given immediately after infection, or a similar dose repeated twice daily. "Delayed treatment" was less effective than immediate treatment. The majority of the deaths occurred before the third day of observation, irrespective of the method of treatment.

Altogether six strains were used, three of which were isolated from guinea-pigs, one from a mouse, and two from human subjects. We are indebted to Prof. W. W. C. Topley for two of the strains, and to Dr. F. A. Knott for one. Suspensions in 5 per cent. mucin of all the cultures were virulent for mice, and gave very similar results with treatment.

A single dose of the sulphonamide produced a slight, but definite, retardation in the time of death.

TABLE II

Treatment of Mice Infected with Bact. aertrycke (*with Mucin**)

Treatment with the sulphonamide.	Approx. number of organisms in infecting dose.	Number of mice (out of groups of 10) dying on each day after infection.							Mice surviving 12 days.
		1st	2nd	3rd	4th	5th	6th	7th–12th	
GUINEA-PIG STRAIN, Q.626									
None.	5,000	5	2	2	..	..	1	..	0
	500,000	9	1	..	..	..	..	..	0
40 mg. twice daily.	5,000	..	..	..	2	..	..	..	8
	500,000	..	..	..	..	3	..	4	3
HUMAN STRAIN, A.6									
None.	50	1	5	..	1	3	..	..	0
	500,000	9	1	..	..	..	..	..	..
25 mg. twice daily.	50	..	..	..	..	..	..	6	4
	500,000	..	..	..	2	5	1	2	0

* Ten mice injected with mucin alone and 10 mice injected with mucin and treated with the sulphonamide all survived.

When administration of the drug was "delayed for five hours" after infection and repeated twice daily, the treated mice survived for only two to three days longer than the controls.

The best effect was obtained with repeated doses of the sulphonamide (Table II), but even here the protection was only temporary. Treatment produced delay in the time of death of mice infected with a large dose of culture (10,000 fatal doses), but the majority of these animals died within a few days of the controls. Only some of the mice infected with approximately one fatal dose could be kept alive for twelve days.

The *Bact. aertrycke* survived for several days in treated mice; although the mice appeared healthy, the blood, spleen, and gall-bladder all gave a growth of the organism seven days after infection, and a positive culture was obtained from the spleen of one animal which had survived for eighteen days.

FRIEDLÄNDER'S BACILLUS

One strain of exceptional virulence was selected from a series sent from the National Collection of Type Cultures through the courtesy of Dr. St. John Brooks. A protocol of a typical experiment made with this strain is appended (Table III). The out-

TABLE III

Treatment of Mice Infected with Friedländer's Bacillus (Strain 12). (Without Mucin)

Treatment with the sulphonamide.	Approx. number of organisms in infecting dose.	Number of mice (out of groups of 10) dying on each day after infection.							Mice surviving 12 days.
		1st	2nd	3rd	4th	5th	6th	7th–12th	
None.	1	..	..	1	5	..	..	..	4
	10	..	1	5	4	..	..	..	0
	100	4	6	..	..	..	..	..	0
	1,000	9	1	..	..	..	..	..	0
25 mg. twice daily.	1	..	..	..	..	..	..	..	10
	10	..	1	..	1	1	..	5	2
	100	..	..	1	4	2	3	..	0
	1,000	..	1	2	7	..	..	..	0
	10,000	..	..	10	..	..	..	..	0
	100,000	1	..	9	..	..	..	..	0
	1,000,000	1	6	3	..	..	..	..	0

standing feature was that frequent doses of the sulphonamide gave temporary protection only. Although, at an early stage of the experiment, mice infected with 10,000 fatal doses appeared healthy, it was only those animals which had received approximately one fatal dose which survived for twelve days.

It is probable that the infection produced in mice by a number of other members of the genus bacterium may be influenced by the sulphonamide. Since this paper was written, slight protection was obtained against a Gram-negative, slow lactose-fermenting bacillus, which was highly virulent for mice. It was isolated by Dr. David Haler from a case of adult dysentery, but does not conform to any of the recognised features of the dysentery group. Dr. Haler informs us that a similar organism is sometimes associated with pyelitis and with infantile dysentery.

PNEUMOCOCCUS

It has already been reported (Buttle et al. 1936) that the sulphonamide has little effect in the protection of mice against infections by the pneumococcus, Type I. In view of the effect obtained against other infections by repeating the doses of sulphonamide at short intervals, experiments on the treatment of pneumococcal infections were resumed.

In the control series, groups of 6 mice were killed within forty-eight hours by infecting doses of approximately 5 pneumococci, Type I. Groups of treated mice (40 mg. twice daily) infected with 5 and 500 pneumococci appeared healthy for the first three days, but some deaths were observed on the fourth day, and by the twelfth day all the mice had died.

The experiment was repeated with another virulent strain of pneumococcus, Type II. The time of death of the treated mice was delayed a little longer than in the case of the infection with the Type I strain.

Rosenthal (1937) studied the effect of the sulphonamide on mouse infections with a number of pneumococcal strains of Types I, II, and III. He reported that 25 to 50 per cent. of the treated mice survived for seven days, and, in the case of one strain, 86 to 100 per cent. of mice were cured. The sulphonamide was injected subcutaneously in emulsion with olive oil. It is possible that strain variation may explain the difference between Rosenthal's results and ours, although another factor may have been the method of administration of the drug.

PASTEURELLA

Preliminary work would suggest that the drug has a temporary and very slight beneficial effect against infections with *Past. pseudotuberculosis* and *Past. septica*. Further experiments are projected, in view of the relationship between these organisms and *Past. pestis*.*

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* *Staphylococcus*.—Since this paper went to press we have tested for virulence a number of strains of staphylococcus isolated from bovine and canine sources by Prof. F. C. Minett. One bovine strain was of exceptional virulence, mice being killed within 48 hours by approximately 50 organisms suspended in mucin. Treated mice that had received 1000 fatal doses survived for upwards of 12 days. In our previous work with a large number of human strains we did not find any organisms of sufficient mouse-virulence to enable us to demonstrate any degree of protection.

Effect of the Sulphonamide in vitro

Only three of the above organisms have been studied so far—viz., *Bact. aertrycke*, *Bact. typhosum*, and pneumococcus.

BACT. AERTRYCKE

The inhibitory effect of *p*-aminobenzenesulphonamide on the rate of growth of this organism in culture medium in vitro is not nearly so great as that found with the streptococcus (Colebrook, Buttle, and O'Meara 1936). An inoculum of approximately 8 organisms of our guinea-pig strain, Q.626, grows in the broth medium described by Wright (1933), and the addition of 0·1 per cent. of the sulphonamide does not inhibit the growth appreciably (see also Nitti, Bovet, and Depierre 1937). If the tubes are incubated anaerobically, however, the addition of 0·1 per cent. sulphonamide inhibits growth for sixteen hours.

If the organisms are grown in defibrinated human blood (G. A. H. B.), and incubated in slowly rotating tubes, it is necessary to use an inoculum of 1,000,000 organisms in 0·5 c.cm. of blood to obtain growth (an inoculum of 100,000 organisms is destroyed by the blood itself). The addition of 0·04 per cent. of the sulphonamide to the blood produces a slight bactericidal effect; 1,000,000 organisms are destroyed, but there is no appreciable effect on an inoculum of 10,000,000 organisms. If the leucocytes are removed from the blood by the method described by Fleming (1926), the addition of 0·04 per cent. of the sulphonamide does not influence the growth of an inoculum of 1,000,000 organisms. With streptococci, on the other hand, the addition of 0·01 per cent. of the sulphonamide considerably delayed the growth of the cocci even in blood from which the leucocytes had been removed. As the sulphonamide produces so little inhibition of growth of *Bact. aertrycke* in culture medium or in deleucocyted blood it seems possible that, in whole blood, it may act by rendering the organisms more susceptible to phagocytosis (see also Long and Bliss 1937).

The effect of the sulphonamide on the growth of *Bact. aertrycke* in vitro appeared to be so slight that experiments have been carried out to determine whether there was any other factor contributing towards the recovery of the treated mice.

The serum taken from two treated mice seven days after infection did not agglutinate *Bact. aertrycke*. Treated mice which had survived for two weeks were just as susceptible as normal mice to a further injection of culture.

In another experiment, a group of six mice was injected intraperitoneally with the soluble polysaccharide prepared by Prof. Topley, from the mouse strain of *Bact. aertrycke* and very kindly sent to us for the investigation; a second group of 6 mice, similarly injected with the polysaccharide was treated with the sulphonamide, and a third group received mixtures of the sulphonamide and the poly-

saccharide. The mice in the treated groups died at the same time as the controls which had not received the sulphonamide.

BACT. TYPHOSUM

The results of tests with this organism were similar to those obtained with *Bact. aertrycke*. In preliminary experiments the drug produced little inhibition of growth in broth, whereas a definite bactericidal action was demonstrable in defibrinated whole blood. An inoculum of 100,000 organisms grew in normal blood, whereas in blood containing 0·04 per cent. of the sulphonamide 1,000,000 organisms were killed, and 10,000,000 were inhibited for 18 hours.

PNEUMOCOCCUS

Although the treatment of pneumococcal infections in the mouse is even less effective than the treatment of *Bact. aertrycke* infections, the inhibition of growth of the pneumococcus, Type I, in a broth medium is nearly as great as that obtained with the hæmolytic streptococcus, and very much greater than that with *Bact. aertrycke*. Thus, 0·01 per cent. of the sulphonamide prevents the growth of 50 pneumococci in broth, while 0·1 per cent. of the sulphonamide is almost without effect on the growth of a smaller inoculum of *Bact. aertrycke*. If, however, the pneumococci are grown in defibrinated human blood (G. A. H. B.), incubated in slowly rotating tubes, the effect of the sulphonamide is only very slight. The cocci grow in twenty-four hours from a very small inoculum—3 organisms—with or without the addition of 0·01 per cent. of the sulphonamide; the growth in the blood containing the sulphonamide, however, is not as rapid as in the controls. The results of experiments in vivo, therefore, correspond more closely with those obtained in vitro in blood than with those in culture medium.

The above results of in-vitro tests are of a preliminary nature, only one strain of each of these organisms having been tried with the blood of two individuals. Further work is clearly necessary on the action of the sulphonamide on several strains of organisms in vitro in the blood of different individuals.

Discussion

The foregoing experiments show that the early oral administration of *p*-aminobenzenesulphonamide prevents or delays the development of septicæmia and death in mice infected with a number of different organisms. An attempt has been made to summarise the available data in Table IV; earlier results with the hæmolytic streptococcus and meningococcus are included for purposes of comparison. It is emphasised that the figures are only approximations

and depend to some extent on the virulence of the organisms used.

It will be seen that there are three grades of protection. In the first group, intensive treatment with the drug merely delays the death of the mice; in the second, frequent doses are requisite if the protection is to be other than temporary; and, in the third, a single dose given immediately after infection produces a maximal effect.

With regard to the rôle of the sulphonamide, evidence has been adduced that the drug has an

TABLE IV

Grades of Protection with p-*Aminobenzenesulphonamide*

—		Approx. number of fatal doses against which mice are protected.	
		For 2 days.	For 12 days.
A. Frequent doses give temporary protection only.	*Bact. aertrycke.*	10,000	1
	Friedländer's bacillus.	10,000	1
	Pneumococcus.	100	0
	Past. pseudo-tuberculosis.	100	0
	Past. septica.	100	0
B. Frequent doses give lasting protection. (Single dose gives temporary protection.)	*Streptococcus hæmolyticus.*	10,000	
C. Single dose gives lasting protection.	Meningococcus.	1,000,000	
	Bact. typhosum.	100–1000	
	Bact. paratyphosum B.	100–1000	

inhibitory effect on the multiplication of small numbers of streptococci and various other organisms in vitro in broth medium and in deleucocyted blood. It is almost certain that this also occurs in the blood in vivo, but it is probable that here the leucocytes also play a part. Long and Bliss (1937) observed a marked degree of phagocytosis by the leucocytes in the peritoneal exudate of mice infected with hæmolytic streptococcus and treated with the sulphonamide; there was no evidence of phagocytosis in the untreated controls. These authors therefore believe that the drug has an important action in injuring the organisms and making them more susceptible to phagocytosis. In our experiments on

Bact. aertrycke and *Bact. typhosum*, we obtained some support for this view; we could demonstrate a slight bactericidal effect for the sulphonamide in whole blood in vitro, but in deleucocyted blood or culture medium there did not appear to be a definite diminution in the rate of growth.

The drug is now being tried in the treatment of hæmolytic streptococcal and also in a few meningococcal infections in man, but the results of the mouse experiments would suggest that it might have an even wider application. In certain infections, the action is probably insufficient to be of clinical value by itself, and, in these, the sulphonamide might possibly be useful in conjunction with other measures—e.g., therapeutic serum in the treatment of pneumonia.

Summary

p-Aminobenzenesulphonamide protects mice against multiple lethal doses of *Bact. typhosum* and *Bact. paratyphosum* B. There is some degree of protection against *Bact. aertrycke*, Friedländer's bacillus, pneumococcus, *Past. pseudotuberculosis*, and *Past. septica*.

A single dose of the sulphonamide gives lasting protection against some of these organisms; repeated doses lead only to delay in the death-time of the mice infected with others.

In no instance are the results of treatment as satisfactory as in hæmolytic streptococcal and meningococcal infections.

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THE CHEMOTHERAPY OF P-AMINOBENZENESULPHONAMIDE AND RELATED COMPOUNDS.

A Thesis

submitted for the degree of Ph.D.

by

MORAG McLEOD ~~or DAVIDSON~~, B.Sc. (Edin.)

May 1939

SECTION 1

REVIEW OF LITERATURE

1. ANIMAL EXPERIMENTS.

With Ehrlich's great discoveries in Germany the foundations of modern chemotherapy were laid, and it was again the work of the German chemists to demonstrate first the chemotherapeutic action of the prontosil groups of compounds. The possible value of azo compounds (compounds containing - N = N - linkage) as bactericidal agents was first demonstrated in 1913 by Eisenberg who showed that diaminoazobenzene or crysoidin would kill the streptococcus. Unfortunately, his successful experiments in vitro could not be repeated in the animal body and it was soon realised that there was an almost unbridgeable gulf between the splendid results in the test tube and the indifferent response in the living infected animal.

Ehrlich drew a comparison between the organic linkages present in certain dye-stuffs with those in certain drugs and this comparison has come to be more than an analogy.

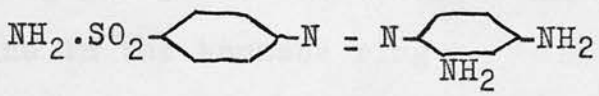
Many years ago the German chemists realised that aniline dyes for certain fabrics had/

had a constitution in which two benzene rings were joined by an azo linkage with a sulphonomide group in the para position on one of them. These azo compounds were originally prepared by Hörlein, Doressel and Kolthe (1909 and 1910) in the Elberfeld laboratories. Some years later the German workers, thinking that this same constitution might confer a destructive affinity for certain microbic protoplasms, infected animals with a variety of pathogenic organisms they had to hand and treated them with aniline dyes of this type.

HAEMOLYTIC STREPTOCOCCAL INFECTIONS

From work such as this arose the discovery by Domagk (1935) that azo compounds containing the sulphonamide group had a remedial action in the streptococcal sepsis of mice. This discovery was the true starting point for the preparation of a new series.

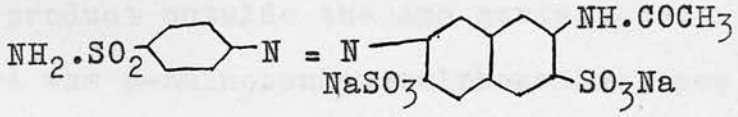
The original prontosil, prontosil red or diaminoazobenzenesulphonamide



was prepared by Mietzsch and Klarer (1932) by diazotising/

diazotising p-aminobenzenesulphonamide and coupling it with m-phenylenediamine. It was introduced in the form of a hydrochloride which is sparingly soluble in water, and later as the free base which is still less soluble. The degree of absorption of both compounds was practically identical. This compound is usually administered orally.

A short time later a sulphonic acid derivative of prontosil red was introduced. This was prontosil soluble, a compound which is given intramuscularly. It has the formula



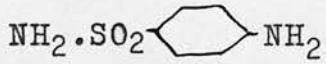
The remarkable action of prontosil was soon recognised to be a fairly general and a moreor less graduated property of a whole class of chemical compounds. These substances are characterised by the presence of a sulphonamide group ($-SO_2 - NH_2$) or of certain other sulphur containing groups as substituents in certain positions in the benzene ring.

Study of toxicological details soon showed the comparative innocuousness of these new drugs. Mice tolerated up to 3 mg. per gram body weight either by mouth or subcutaneously.

Moreover, when given in fairly large amounts the dyes were found to penetrate into all the tissues a fact which probably explains their potent action on streptococci localised in the most varied situations in the body.

The work at the Elberfeld laboratories by Domagk was confirmed by Levaditi and Vaisman (1935, 1936) at the Pasteur Institute and also by Girard (1936).

Working independently of the Elberfeld investigations, Tréfouël, Tréfouël, Nitti and Bovet (1935) prepared a colourless antistreptococcal product outside the azo series. This compound was p-aminobenzenesulphonamide, now frequently called sulphanilamide. It has the simple formula

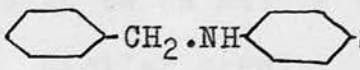


These French investigators were the first to show that the azo grouping was unnecessary and that this simple compound was equally effective in curing haemolytic streptococcal infections in both mice and rabbits.

In a series of articles (1936, 1937) the French workers gave further accounts of their experiments on many substances allied to prontosil and/

and on a large number of derivatives of p-amino-benzenesulphonamide. They found that if the m-phenylenediamine portion of the prontosil molecule was replaced by allied groups the anti-streptococcal activity was little altered, but if the aminosulphonamide portion was replaced the activity was found to disappear. From these results it seemed possible that the active agent in prontosil was the simple p-aminobenzenesulphon-amide, and that the latter substance was formed from prontosil in the animal body by reduction at the azo linkage.

The fact that p-aminobenzenesulphonamide is active in streptococcal infections in mice was confirmed by Goissidet et al (1936) who also showed that the benzyl derivative of p-aminobenzene-sulphonamide

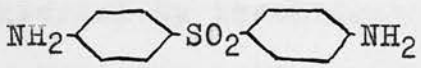


has a similar though less marked curative effect on haemolytic streptococcal infections of mice.

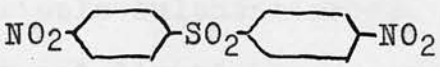
The first published contribution by British workers came from Buttle, Gray and Stephenson (1936) who tested p-aminobenzenesulphon-amide against several serological types of the haemolytic/

haemolytic streptococcus and found that the compound would protect mice against 50,000 lethal doses of the organism. The anilide of sulphanilic acid was found to be as active as the amide though sulphanilic acid itself was almost inactive.

Buttle and Stephenson (1937) have shown that the mouse protective action is much more pronounced against streptococcal infections in mice in the case of 4.4' diaminodiphenylsulphone and certain of its derivatives.

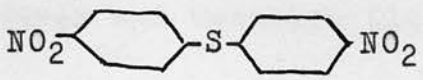


This compound though 25 times as toxic as sulphanilamide was shown to be 100 times as active while 4.4 dinitrodiphenylsulphone



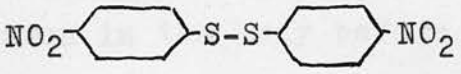
was found to be as active and slightly less toxic.

Similar results were obtained independently by Fourneau et al (1937) who have supplemented this work by investigating other sulphur products, namely diphenylsulphide and diphenyldisulphide derivatives. They found that 4.4' dinitrodiphenylsulphide

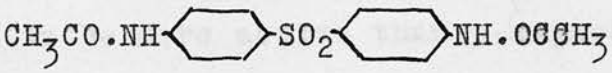


was/

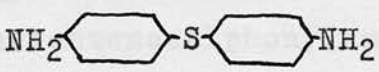
was about 4 times as active as sulphanilamide and that 4.4'dinitrodiphenyldisulphide



was about 4 - 8 times as active. Continuing their work on the protective action of organic sulphides and sulphones against diverse experimental septicaemias, the French investigators obtained good results in streptococcal and pneumococcal infections in mice with 4.4' diacetyldiaminodiphenyl-sulphone



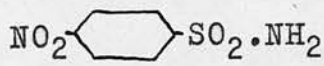
Gley (1937) confirmed these results and contributed further material by investigating the action of p-acetylaminobenzenesulphinic acid and p-acetylamino-benzenethiophenol. He found that these compounds had an antistreptococcal action comparable with the simple sulphanilamide. In a further paper Gley and Girard (1937) record their results on the therapeutic action of 4.4' diaminodiphenyl-sulphide or thioaniline



against the streptococcus in the mouse. This compound also showed a greater activity than p-aminobenzenesulphonamide.

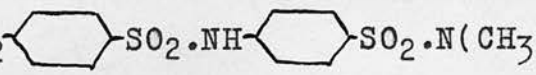
Mayer and Oechslin (1937) realising that in/

in the case of sulphanilamide there is a possibility of oxidation in the body before direct action on the organisms occurs, tried the antistreptococcal activity of the following compounds; p-p'-hydrazobenzenesulphonamide, p-nitrobenzenesulphonamide, p-nitrosobenzenesulphonamide and p-sulphamidobenzene hydrazine. The last two compounds were completely inactive but p-nitrobenzenesulphonamide

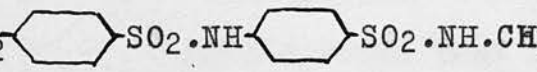


was found to be more active than p-aminobenzenesulphonamide. p-p'-hydrazobenzenesulphonamide was completely inactive.

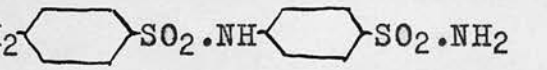
A paper appeared by Domagk (1937) describing the activity of the "uliron" group of compounds.

(1) 

p-aminobenzenesulphonylaminobenzenesulphondimethylamide or uliron.

(2) 

p-aminobenzenesulphonylaminobenzenesulphonmethylamide.

(3) 

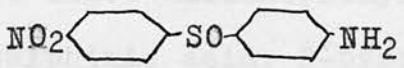
p-aminobenzenesulphonylaminobenzenesulphonamide.

These compounds previously mentioned by Buttle, Gray and Stephenson (1937) and by Rosenthal Bauer/

Bauer and Branham (1937), were found to be all equally effective in haemolytic streptococcal infections of mice and were comparable to sulphanilamide and prontosil in therapeutic activity.

Girard, Raymond Richard (1937) reported that from mouse experiments they were able to show a high curative activity with a series of para-substituted aromatic sulphoxides containing hydroxyl, amino and nitro groups, not only against streptococcal but also against gonococcal infections.

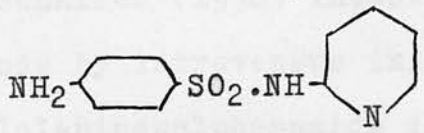
A later paper by Levaditi, Girard and others (1937) gave general indications of the type of compound showing antistreptococcal activity. They carried out tests on 75 compounds, and of these found that the most active was 4-nitro-4'-aminobenzenesulphoxide



Working with pyridine derivatives Kolmer, Brown and Raiziss (1937) found that 2.2'pyridyl-sulphide-dihydrobromide produced an effect similar to prontosil and sulphanilamide against streptococcal infections in rabbits.

The introduction of pyridine into the molecule/

molecule resulted in the discovery of the activity of 2-sulphanilylaminopyridine, M. & B. 693 or "Dagenan"



which was tested in experimental infections by Whitby (1938) who examined a large number of newly synthesised drugs prepared by May and Baker's research laboratories. This compound is highly active against streptococcal infections in a dose as low as 1 milligram per 20 gram mouse, and it is also active against the pneumococcus, and the gonococcus.

Continuing their investigation of substances allied to 4.4'-diaminodiphenylsulphone in streptococcal infections of mice Buttle et al (1938) found that diacetamidodiphenylsulphone, p-acetamidobenzene sulphinic acid, 2-pyrrolidone-5-carboxylamidobenzene-4-sulphinic acid were active whereas tetrabenzyldiaminophenylsulphone, 4.4'dichlordiphenylsulphone and 4.4'dihydroxyldiphenylsulphone were inactive.

Crossley et al (1938) found many compounds/

compounds of the "Uliron" or disulphanilamide type to be at least equal to the original p-aminobenzenesulphonamide in antistreptococcal activity.

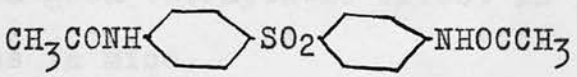
Schaffer (1938) investigating various new compounds by intravenous injection has found that phenylalaninesulphonamide is superior to p-aminobenzenesulphonamide in experimental haemolytic streptococcal infections of rabbits.

PNEUMOCOCCAL INFECTIONS

Experimental studies carried out with sulphanilamide have shown that this compound is relatively inactive against the pneumococcus, as compared with its activity against the haemolytic streptococci. The original impression given by Domagk (1935) was that prontosil red had some action on pneumococcus Type III but none on Types I and II; later (1936) he found that sulphanilamide was more effective against pneumococci than was prontosil. Buttle et al (1937) stated that sulphanilamide merely delayed death in mice infected with pneumococci and Rosenthal (1937) reported that in mice, sulphanilamide had some activity against 7 strains of pneumococcus (Types I, II and III) but indicated that there was considerable variation in susceptibility to different strains.

Cooper, Gross and Mellon (1937) found some effect against 10 lethal doses of a highly virulent Type III coccus inoculated subcutaneously into mice. Cooper and Gross (1937) found also some protection against the intrathecal injection of Type III pneumococcus in rats as well as against two strains of Type II and of Type I, while Schmidt (1937) found sulphanilamide effective against 10 lethal doses of Type XIV pneumococcus. Whitby (1937) found almost no protection with sulphanilamide against 10,000 lethal doses of Type I pneumococcus. It would seem that although sulphanilamide has a certain effect against a small number of lethal doses of the pneumococcus it is notable to control the heavy infection in mice.

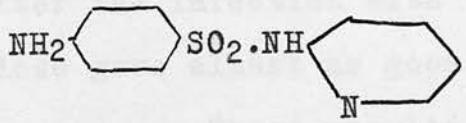
Fourneau, Tréfouël, Nitti and Bovet (1937) obtained good effects in the pneumococcal infections of the mouse with diacetyldiaminodiphenylsulphone.



One milligram of this compound showed an action equal to 10 milligrams of sulphanilamide and the tolerated dose was very much greater.

Girard and Vaisman (1938) reported a small antipneumococcal activity from 4.4'diacetyl-dihydroxydiphenylsulphone.

Whitby (1938) brought forward the first really effective antipneumococcal compound. This was 2-sulphanilylaminopyridine or M. & B. 693



From his experiments Whitby found that M. & B. 693 protected mice effectively against 10,000 lethal doses of pneumococcus Type I and afforded considerable protection against 10,000 lethal doses of other types of pneumococci.

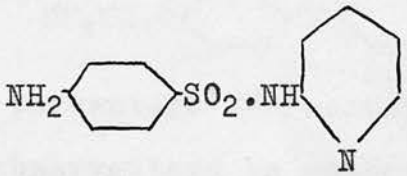
Buttle et al (1938) reported that 4.4' diaminodiphenylsulphone had some action on pneumococcal infections of mice but the effect was rather to delay death than to cure completely. In a later paper (1938) they reported that the benzylidene derivative (Schiff's base) of the sulphone produced a good therapeutic effect in pneumoccal infections in mice.

MENINGOCOCCAL AND GONOCOCCAL INFECTIONS

Buttle, Gray and Stephenson (1936) were the/

the first group of workers to describe the results of animal investigations on mice infected with the meningococcus. They were able to demonstrate complete protection of mice against a dose of one million meningococci. Proom (1937) supplemented these results and found that the dose must be given immediately after the infection with the organisms and that one dose gave almost as good a protection as repeated doses. These results have been confirmed by Levaditi and Vaisman (1937) in France and by Weinberg, Mellon and Shin (1937) in America.

Rosenthal et al (1937) showed that disulphanilamide or p-aminobenzenesulphonylamino-benzenesulphonamide was more effective than sulph-anilamide against meningococcal infections of mice, whilst Whitby (1937) found 4.4'diaminobenzenesulph-onanilide tartrate as effective as p-aminobenzene-sulphonamide. Whitby (1938) has also demon-strated the activity of M. & B. 693 or 2-sulphanilyl-aminopyridine



in experimental meningococcal infections.

The gonococcus is almost non-pathogenic

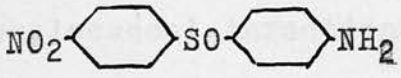
to/

to animals and it is only fairly recently that true gonococcal infections have been produced in mice.

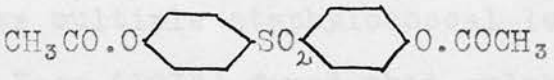
Long and Bliss (1938) have reported that experimental gonococcal peritonitis and septicaemia can be cured by sulphanilamide.

Carpenter, Hawley and Barbour (1938) infected mice with lethal doses of gonococcal toxin and found that they were saved from death by adequate doses of sulphanilamide.

Levaditi and Vaisman (1937) found that 4-nitro-4'-aminodiphenylsulphoxide would protect mice against the gonococcal endotoxin.



They also found that animals so treated did not acquire antigonococcal immunity. The same group of workers infected mice with both the meningococcus and the gonococcus and showed that treatment with 4.4'diacetoxydiphenylsulphone



secured a high percentage of recoveries.

The observations on gonococcal infections of mice will suffice to show that sulphanilamide and a number of sulphonomide compounds are effective against gonococcal infections in experimental animals.

Although the present discussion is limited to reports of animal experiments it is of interest to note that M. & B. 693 or 2-sulphanilylaminopyridine has been found clinically to have outstanding antigonococcal action in addition to its antistreptococcal and antipneumococcal effects. At the same time, as far as the writer is aware, there have been no reports of successful animal experiments with this compound on the gonococcus.

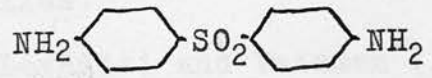
STAPHYLOCOCCAL INFECTIONS

Domagk (1935) obtained fairly good results with prontosil as a chemotherapeutic agent against staphylococcal infections of rabbits.

Buttle et al (1937) found that treated mice which were infected with 100 lethal doses of the staphylococcus survived for several weeks, but at the end of this time many animals died off, and on post-mortem examination almost all the animals were found to have multiple staphylococcal lesions.

De and Basu (1938) found that sulphanilamide protected mice against the staphylococcus but they obtained much better results with a combination of antitoxic and antibacterial staphylococcal serum along with sulphanilamide treatment.

Buttle et al (1938) mentioned that 4.4' diaminodiphenylsulhpone was effective in staphylococcal infections of mice.



Domagk (1937) claimed to have obtained very much better results than had previously been shown using "Uliron" or p-aminobenzenesulphonylaminobenzenesulphondimethylamide against staphylococcal septicaemias of mice.

COLI-TYPHOID INFECTIONS

Buttle et al (1937) investigated the therapeutic efficiency of sulphanilamide in infections in mice produced by the typhoid.bacillus and the paratyphoid bacillus and found that the drug would protect mice against about 100 lethal doses of these organisms. In the case of Bacillus Aertrycke and Friedlander's pneumobacillus frequent doses of sulphanilamide only gave a temporary protection. However, mice infected with 10,000 lethal doses of the causative organism of Sonne Dysentery were completely protected by sulphanilamide.

Levaditi and Vaisman (1935) were unsuccessful in obtaining action in mice with prontosil red therapy against Bacillus paratyphosus B and against Friedlander's infections in mice.

Bürgers (1937) was similarly unsuccessful in obtaining protection in mice against Friedlander's pneumobacillus.

Levaditi and Vaisman (1938) reported the successful antiendotoxic action of various sulphonamide and sulphoxide compounds towards the endotoxin of both Flexner's and Shiga's dysentery bacillus when the endotoxin was injected into mice intravenously.

TUBERCLE INFECTIONS

Rich and Follis (1938) reported that sulphanilamide exerted a striking inhibitory effect on the development of experimental tuberculosis in guinea pigs. Buttle and Parish (1938) confirmed this result but found that p-aminobenzenesulphonamide had no effect on rabbits infected with bovine tuberculosis. Smithburn (1938) found that there was no change in the survival time of tuberculosis infected animals when treated with sulphanilamide.

MISCELLANEOUS INFECTIONS

Domagk (1937) obtained successful results treating mice infected with the organism of gas-gangrene (Bacillus Welchii) with the uliron group of compounds (see page 8).

Sulphanilamide therapy against Pasteurella Pseudotuberculosis and against Pasteurella Septica was investigated by Buttle et al (1937) who obtained only a temporary and very slight beneficial effect in the treated as compared with the control group.

Levaditi and Vaisman (1935) obtained negative results using prontosil red therapy against Pasteurella infections. Recently (1938) Levaditi and Reinié have found that sulphanilamide and also certain sulphone derivatives are active against Pasteurella Avidica infections of mice and fowls.

Chin (1938) has reported that daily oral administration of 100 milligrams of sulphanilamide to guinea pigs infected with lethal doses of Brucella Abortas and Brucella Suis resulted in complete protection from the infections.

Bürgers (1937) was unsuccessful with prontosil therapy against pertussis infections in mice.

Cruikshank (1938) found that both sulphanilamide and M. & B. 693 when given orally or by injection are without effect on the course of experimental pertussis infections in mice (infected by the intranasal route).

Coggeshall (1938) found that sulphamilamide treatment/

treatment by mouth or injection prevented or cured malaria in monkeys caused by intraperitoneal injections of Plasmodium knowlesi. Similar experiments with p-aminobenzenesulphonylaminobenzene-sulphonamide gave negative results. Neither drug was of any value against Plasmodium cathemerium in canaries or Plasmodium cophurae in chicks. Das Gupta and Chopta (1938) confirmed these results using soluseptasine to cure monkeys infected with Plasmodion knowlesi. However, Buttle et al (1938) were unsuccessful with sulphanilamide therapy against malaria in canaries.

Niven (1938) came to the conclusion that although prontosil had some action on malarial parasites, especially on Plasmodium falciparum, it has no place in the treatment of malaria owing to its moderate efficiency, high toxicity and cost.

Many attempts have been made to apply sulphanilamide therapy to the virus diseases but as yet there are few reports of successful therapy. There are three accounts of negative results in attempts to cure or prevent the occurrence of poliomyelitis in monkeys by dosage of sulphanilamide. These come from Kelson (1937), McKinley, Acree and Meck (1938) and from Toomey and Takacs (1938).

Chemotherapeutic experiments on the virus causing lymphogranuloma inguinale have been reported from MacCallum and Findlay (1938) who found that both sulphanilamide and a glucose derivative of 4.4'diaminodiphenylsulphone protected a large percentage of mice against the virus. These results have been confirmed by Levaditi (1938) and Bär (1938).

Marcus and Necheles (1938) found that sulphanilamide treatment was effective against the virus of distemper in the course of an epedemic of distemper in the laboratory kennels. Whitney and Dickerson were unable to find that prontosil, sulphanilamide or sodium sulphanilyl sulphanilate (the sodium salt of p-aminobenzenesulphonylaminobenzenesulphonamide) had any action on canine distemper. These results were in direct opposition to those of Dochez and Slanetz (1938) who claimed that sodium sulphanilyl sulphanilate exerted a curative action in both dogs and ferrets. MacIntyre and Montgomerie (1938) have not been able to confirm these results.

Oakley (1938) tried the effect of many sulphur drugs on the virus of influenza; he found that/

that there was a slight curative effect with 4.4'-diaminodiphenylsulphone glucoside.

Boyland (1938) has reported that 4.4'-diaminodiphenylsulphoxide and sodium sulphanilyl sulphanilate retard the growth of spontaneous mammary cancer in mice. Amongst other compounds tested Boyland found that sulphanilamide itself also seemed to exert an effect on mammary cancer. Findlay and MacCallum (1938) found that lymphocytic chloriomeningitis in mice, yellow fever and rift valley fever infections in mice were unaffected by prontosil and allied drugs.

Levaditi (1938) reported that aromatic sulphur compounds of the sulphonamide, sulphone or sulphoxide type are valueless against diseases caused by neurotrophic viruses.

The above account of the results hitherto published in the action of sulphur containing compounds on various experimental animal infections does not claim to be exhaustive. The literature which is already very extensive is multiplying very rapidly and it is not possible to do more than indicate the most important results.

SECTION II

MODE OF ACTION OF SULPHONAMIDE COMPOUNDS ON BACTERIA

The manner in which all these drugs exert their action has been the subject of much study. Were it clearly understood how they act it would be possible to forecast activity from the theoretical aspect and evolve some method of preliminary trial less laborious than the empirical assay on animals.

At the present time comparatively little progress has been made in the discovery of the fundamental principles underlying chemotherapeutic action or of the detailed mechanism whereby active drugs cure animals infected with particular diseases. In the case of the sulphur containing compounds a large amount of work has been done with the object of elucidating the mode of action but many points are still obscure. Two main questions present themselves; first whether the compounds act directly on the bacteria or whether their effect is indirect, for example, through stimulation of leucocytes or neutralisation of toxins, and second whether particular compounds are themselves the active/

active agents or whether they are first converted in the body by suitable chemical change into the really active compounds.

Various authors have adduced evidence in favour of the view that these sulphonamide compounds have an indirect action. Levaditi et al (1935) claimed some neutralisation of streptococcal toxin by the prontosil compound. Levaditi and Vaisman (1937) brought forward further evidence to support the theory of the neutralisation of certain microbic toxins by sulphur benzene derivatives when they showed the action of sulphides, sulphones, and sulphoxides on the gonococcus. Their experiments on the antigonococcal action of 4 nitro-4-aminodiphenylsulphoxide demonstrated anti-endotoxic activity. Further the mice having survived an intraperitoneal inoculation of the gonococcal endotoxin acquired an antigonococcal immunity. It seemed that the benzene sulphur derivatives neutralised the endotoxin and at the same time increased its antigenic power.

Osgood (1938) carried out experiments with bone marrow in order to try to elucidate the mechanism of the action of sulphanilamide. He maintained that the major effect was the neutralisation/

neutralisation of the toxins of the streptococcus, and that the drug had no direct effect either on the organisms or on the phagocytosis of the bacteria by the leucocytes. For this theory there is little evidence, as many antiseptics have the power of neutralising the streptococcus haemolysin, and Osgood used the disappearance of haemolysin from streptococcal cultures as the chief argument in favour of his theory.

Investigating the bacteriostatic action of p-aminobenzenesulphonamide on the haemolytic streptococcus Finklestone-Sayliss, Paine and Patrick (1937) showed that the bacteriostatic action is preceded by a phase of growth stimulation which is more pronounced in young cultures than in cultures which have passed through the logarithmic phase of growth. Sulphanilamide did not appear to modify the activation of polymorphonuclear leucocytes but stimulated the phagocytic activity of reticuloendothelial cells of rabbits and the production of polymorphonuclear leucocytes by the bone marrow. Further, Finklestone-Sayliss et al. found that sulphanilamide was more soluble in the fatty envelope that can be separated from the haemolytic/

haemolytic streptococcus that it is in aqueous solution. At the same time it is generally agreed that these compounds have not a very marked bactericidal action in vitro, and so a simple direct lethal action on the organism seems to be excluded. However, there is a large amount of evidence which shows that in the presence of these compounds the growth of many bacteria is inhibited even although they are not actually killed. The idea has therefore been put forward that the action of the compound is primarily a direct bacteriostatic one and that the leucocytes are then able to deal with the organisms in their resting and perhaps enfeebled condition. In support of this view Colebrook, Buttle and O'Meara (1936) found that blood or serum mixed with prontosil in vitro had no action on the streptococcus whereas blood or serum mixed with sulphanilamide had a definite bacteriostatic and bactericidal power. They also showed that the streptococcus could grow in deleucocyted blood in vitro at the same rate for the first four hours whether sulphanilamide was present or not; afterwards there was no further increase in the number of organisms in the sulphanilamide specimen but the cocci/

cocci continued to multiply in the control. In the whole blood there was a similar initial increase in numbers after which the count fell until the blood became sterile.

These properties are different from those of the ordinary antiseptics which kill microbes well in water, less well in serum and are even less effective in blood. As shown by Buttle et al with sulphanilamide the reverse effect is the case. Nitti and Bovet (1937) confirm these results with both the streptococcus and the pneumococcus, though it is known that sulphanilamide gives very poor chemotherapeutic results in animals experiments with the pneumococcus as the infecting organism. Nitti and Bovet (1937) also showed that the growth of staphylococci, Bacillus Coli and Bacillus Typhi murium (Bacillus Aertrycke) was not influenced by sulphanilamide but there was some bacteriostatic action against a Brucella Abortus culture.

Fleming (1938) carried out the most convincing experiments with the streptococcus and the pneumococcus and M. & B. 693. Using whole blood or deleucocyted blood as the test medium Fleming showed that the action was bacteriostatic and not bacteriocidal at therapeutically obtainable concentrations/

concentrations of the drug and that leucocytes are necessary for bacterial destruction. He also showed that the effect of the drug was enhanced by the addition of specific immune serum to the test medium.

Studying the mode of action of sulphanilamide in experimental streptococcus empyema Gay and Clark (1937) came to the conclusion that the bacteriostasis produced by sulphanilamide appeared to facilitate the normal phagocytosis by the large mononuclear cells. In confirmation of a direct bacteriostatic action are the observations of Whitby (1938) which are to the effect that the capsule of the organisms tends to be damaged in presence of the compound. (This is doubted by Long (1939)). It was early noted by Levaditi et al (1935) that sulphanilamide had some action on streptococcal capsules and so favoured natural phagocytosis in the animal body. These results may be taken as a sign that there is some disturbance in the defence mechanism of the bacteria presumably rendering them more liable to phagocytic destruction.

With regard to the second main question at issue concerning the identity of the really active agent, we have already described the experiments/

experiments of Nitti and Bovet (1935) which led them to the conclusion that when the dyestuffs of the prontosil group were administered to animals they were reduced to p-aminobenzenesulphonamide and that this was the really active material. There seems to be little doubt that they are correct in considering that in the case of these dyestuffs a preliminary reduction occurs in the body yielding sulphanilamide which is known to be active chemotherapeutically. However, it may be that sulphanilamide itself undergoes further change with the formation of some unknown very highly active derivative. It is worth noting that p-nitrobenzenesulphonamide is 4 or 5 times as active as sulphanilamide itself. Mayer (1937) has prepared the intermediate compounds the hydroxylaminobenzenesulphonamide and the nitrosobenzenesulphonamide, and this author is inclined to the view that the p-hydroxylaminobenzenesulphonamide is formed in the body from p-aminobenzenesulphonamide or that a nitrobenzenesulphonamide is formed and it is this which produced the therapeutic effect. However, this view cannot be said to be definitely proved. Even although we previously agree that p-aminobenzenesulphonamide acts directly as a therapeutic agent/

agent it does not necessarily follow that all active compounds of the group are first converted into this particular derivative. Indeed the fact that compounds such as 4-4'dinitrodiphenyl-sulphone and 4-4' diaminodiphenylsulphone have a very chemotherapeutic activity seems quite incompatible with the view that sulphanilamide is the only active compound for the conversion of 4-4'diaminodiphenylsulphone in the body seems to be extremely improbable. In the light of facts such as these it seems probable that chemotherapeutic action may be a property of a fairly large group of compounds containing sulphur and the possibility is not excluded that even certain azo dyestuffs may have a direct action as well as that after their reduction to the simple sulphanilamide. This may account for the fact brought forward by Domagk for direct action who stated (1937) that he does not believe that activation of prontosil is accompanied by its reduction to sulphanilamide. Though he does not offer an alternative explanation Domagk gives many reasons for his denial of this hypothesis amongst them the discrepancy between the in vivo and the in vitro results and the variability of the action os sulphanilamide and related compounds on different/

different animals. The German investigators have also reported that they consider p-nitro-benzenesulphonamide representing the highest form of oxidation of the nitrogen in the para position to be the active agent. They assume that this compound is reduced to sulphanilamide in the animal body.

Whatever criticism may be made of these numerous claims it is obvious that if the remarkable specific action of these drugs is to be understood the question concerning specific stimulation of the immunity mechanism of the body or of the drug being identical with specific antibodies has to be explored

SECTION II

EXPERIMENTAL

At the Wellcome Physiological Research Laboratories, after Dr Buttle's successful work on the streptococcus, the investigation was extended by the writer and others to numerous other organisms, and p-aminobenzenesulphonamide was found to have a certain antibacterial activity for the meningococcus, Bacillus Typhosus, Bacillus Paratyphosus B, Bacillus Aertrycke, the Pneumococcus and for Friendlander's Pneumobacillus. The results of this investigation were published in the Lancet (1937), and the work has been continued and extended in the Royal College of Physicians' Laboratory, Edinburgh, and in the Bacteriolgy Department, University College, Dundee, using compounds prepared in the Chemistry Department of the Royal College of Physicians' Laboratory by Dr Kermack and assistants (Kermack, Spragg & Tebrich, 1939). The results of the work in Edinburgh and Dundee forms the material for the present thesis.

The experiments described in the following pages fall naturally into two sections, the first consists/

consists of a considerable number of experiments in mice in the course of which the toxicity and the chemotherapeutic action of 22 compounds have been determined. The mice were infected with strains of the streptococcus, the pneumococcus, the staphylococcus and Bacillus Aertrycke and the various compounds were administered under the conditions described in detail later. Every compound was not tested on each organism, the gaps being due chiefly to the small quantities of certain compounds available. The results of these experiments are summarised in Table I (page 47).

The main object of the second group of experiments has been to elucidate the mode of action of the sulphur containing compounds. A number of different lines of approach were followed. The action in vitro of the compounds on the organisms was examined in the hope that when all the results obtained on the four different organisms and over a considerable range of chemicals, were examined some relation might be detected between them and the chemotherapeutic actions in vivo. The results of the in vitro experiments are shown in Table II (page 150) and are discussed in Section III.

As it seemed unlikely that a simple direct action of compound on the bacteria was responsible for the chemotherapeutic activity, it was thought that if the conditions in the test tube were made to resemble more closely those of the body tissues, the compounds might then have a more pronounced bactericidal effect. Amongst the obvious experimental factors which could be varied were the hydrogen ion concentration and the oxidation-reduction potential of the medium. These observations were therefore carried out with the object of determining how far the bactericidal action of p-aminobenzenesulphonamide itself was influenced by changes of these factors. These experiments are discussed on pages 236 - 247.

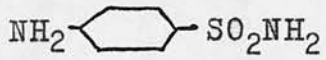
Another possibility is that sulphanilamide is transformed in the body to some much more active compound. As mentioned in the introduction it has been suggested that it may be oxidised to p-hydroxybenzenesulphonamide. Experiments were therefore performed to find out whether general oxidation of p-aminobenzene-sulphonamide in presence of the enzymes of liver tissue or in presence of manganese/

manganese salts may not transform it to highly active products of this kind. These experiments are described on pages 248 - 250.

The results of the original investigations are discussed in Section III page 251.

COMPOUNDS INVESTIGATED

1. p-aminobenzenesulphonamide



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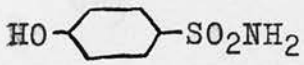
11. p-hydroxybenzenesulphonamide



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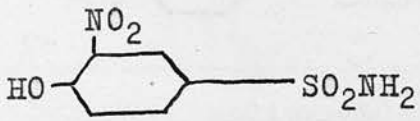
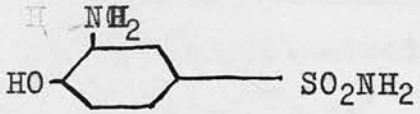
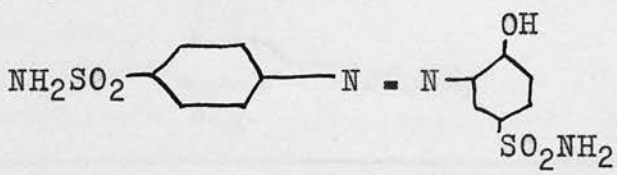
111. 3-nitro 4-hydroxybenzenesulphonamide



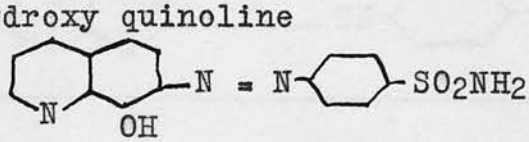
1V. 3-amino 4-hydroxybenzenesulphonamide



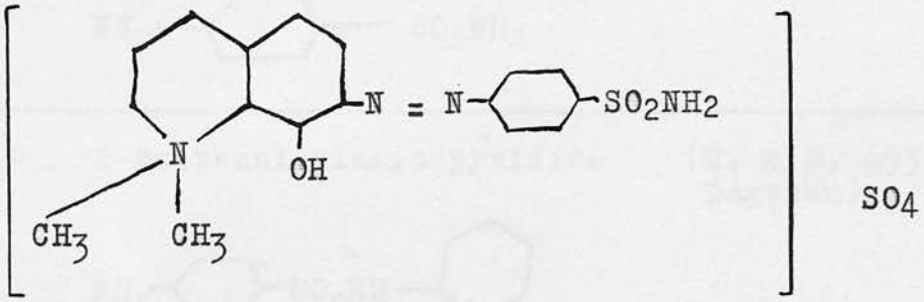
V. p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide



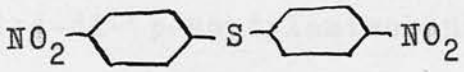
Vl. p-aminobenzenesulphonamide coupled with 8-hydroxy quinoline



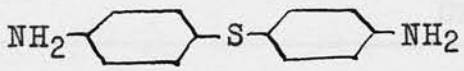
VII. p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate



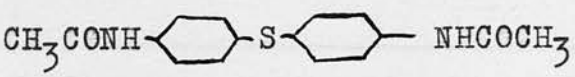
VIII. 4:4'-dinitrodiphenylsulphide



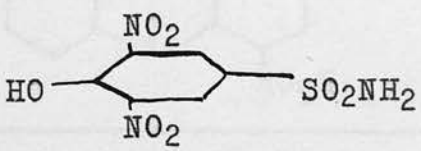
IX. 4:4'-diaminodiphenylsulphide



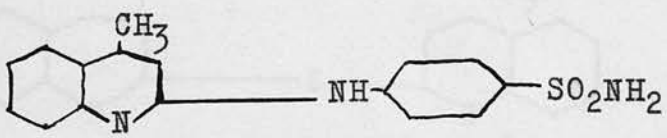
X. 4:4'-diacetyldiaminodiphenylsulphide



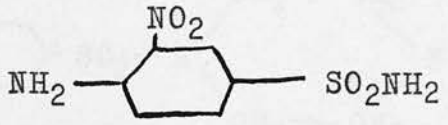
XI. 3:5-dinitro-4-hydroxybenzenesulphonamide



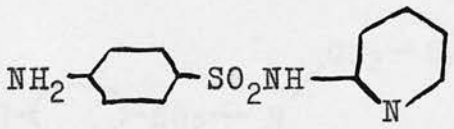
XII. methylquinolylaminobenzenesulphonamide



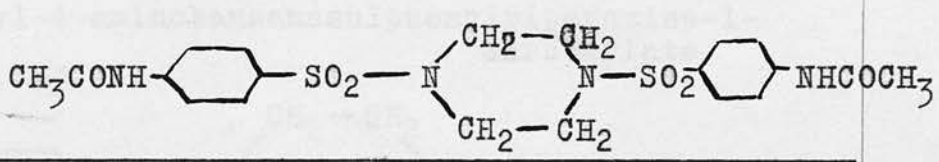
XIII. 3-nitro-4-aminobenzenesulphonamide



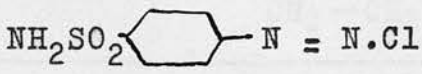
XIV. 2-sulphanilylaminopyridine (M. & B. 693, Dagenan)



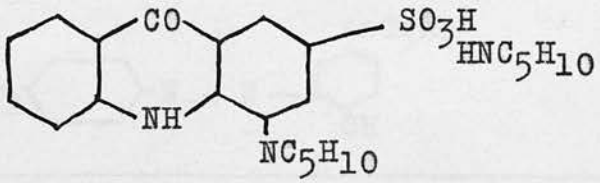
XV. 1:4-di-(p-acetylaminobenzenesulphonyl) - piperazine



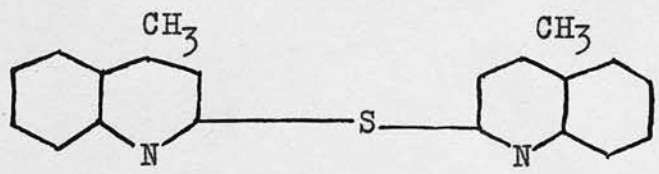
XVI. sulphonamidobenzenediazonium chloride



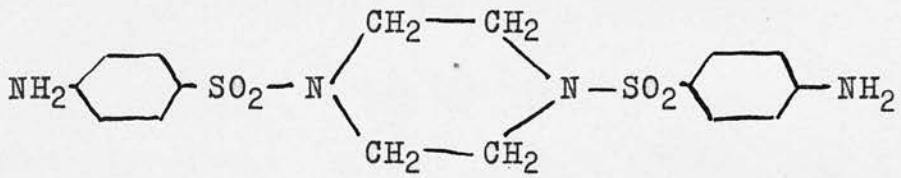
XVII. piperidine-1-piperidino-acridone-3-sulphonate



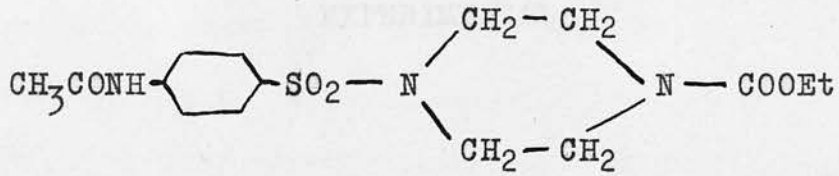
XVIII. 4:4'-dimethyldiquinolyl-2:2'-sulphide



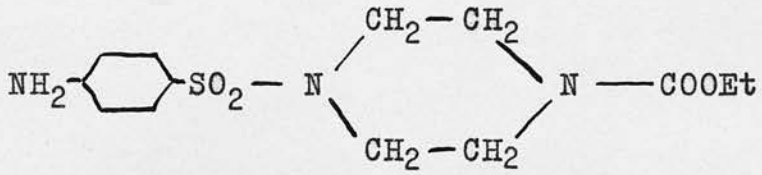
XlX. 1:4-di-(p-aminobenzenesulphonyl)-piperazine



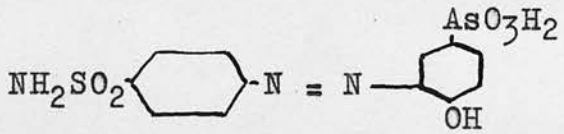
XX. ethyl-4-acetylaminobenzenesulphonylpiperazine - 1-carboxylate



XXl. ethyl-4-aminobenzenesulphonylpiperazine-1-carboxylate



XXll. p-aminobenzenesulphonamide coupled with p-hydroxyarsonic acid



SECTION II

EXPERIMENTAL

PART I - IN VIVO EXPERIMENTS.

PROCEDURE

The mouse was selected as a suitable animal for these experiments. It is a small animal; easy to handle; and many bacterial organisms when injected intraperitoneally into mice are capable under certain conditions of causing the death of the injected animals within 24 - 48 hours. Thus, the chemotherapeutic value of various chemical preparations against a particular organism is fairly easily assessed. Mice of approximately 20 grams weight were used in these experiments and each mouse was weighed before injection.

The mouse was held in the supine position by an assistant who grasped it firmly by the tail and the back of the neck while 1 ml. of a suspension of organisms was injected intraperitoneally. Immediately after the injection of the organisms, a slender blunt cannula attached to a record syringe was introduced into the animal's mouth and down the oesophagus for some distance (see Fig. 1). 1 ml. of the solution or suspension of the compound under examination was then injected into the alimentary tract of the animal. As many of these compounds are very quickly eliminated from the animal body/

body, in order to keep the required concentration of the agent in the body, it was necessary to feed the mice again after 5 or 6 hours and to continue the feeding daily (or twice daily) until the animals had completely recovered from the disease (about one week or shorter if the compound was active) or as long as they survived. If the compound was extremely active, as in the case of p-aminobenzene-sulphonamide against the haemolytic streptococcal infection, the treated mice showed few signs of disease and recovered completely. The control, untreated animals died of a haemolytic streptococcal infection within 24 hours.

In each case the course of the disease was followed for two weeks or longer.

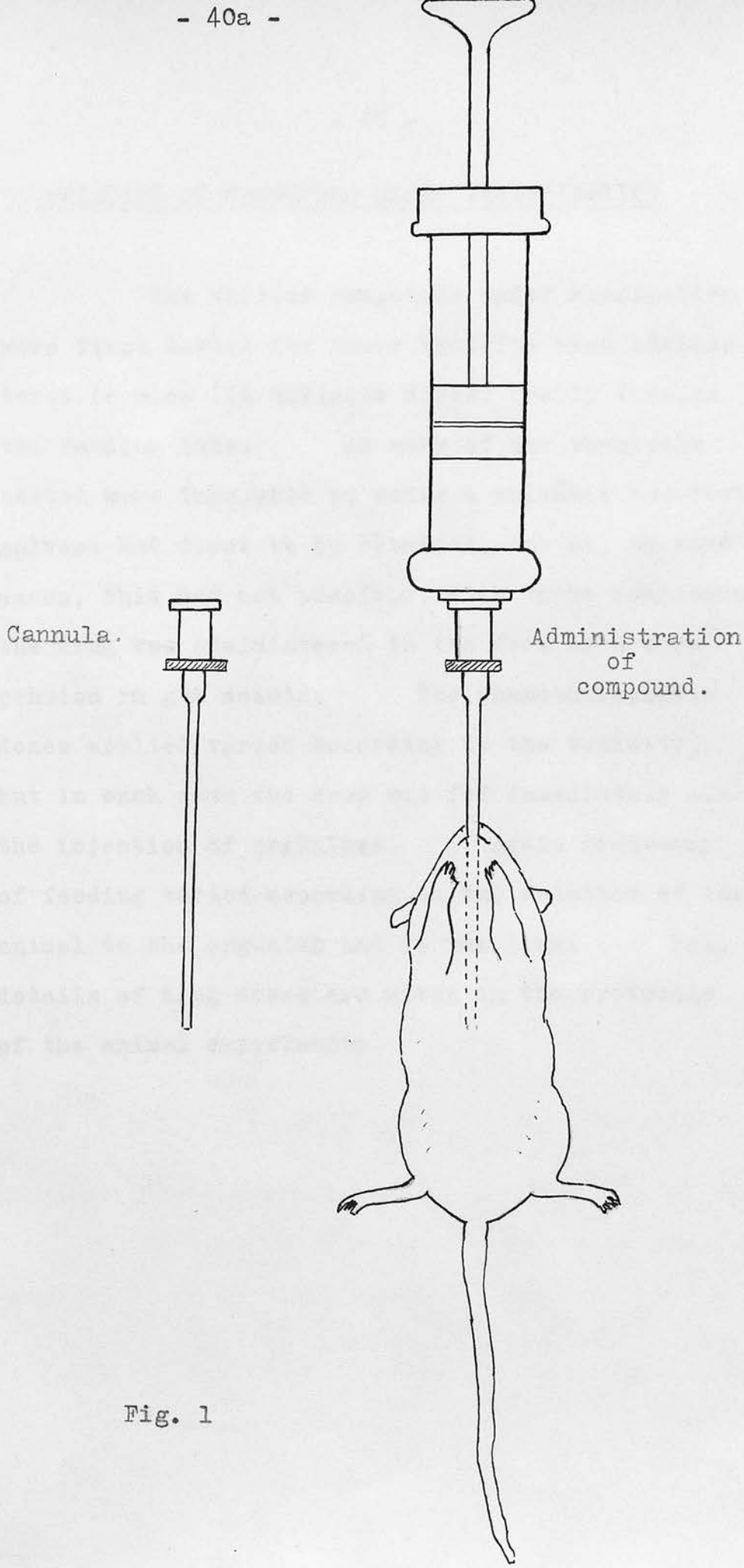


Fig. 1

TOXICITY OF COMPOUNDS UNDER INVESTIGATION

The various compounds under examination were first tested for their toxicity when administered to mice (in definite doses) orally through the feeding tube. As many of the chemicals tested were insoluble in water a suitable non-toxic solvent had first to be obtained, but as, in some cases, this was not possible, with these compounds, the drug was administered in the form of a suspension in gum acacia. The chemotherapeutic doses applied varied according to the toxicity, but in each case the drug was fed immediately after the injection of organisms. Again frequency of feeding varied according to the reaction of the animal to the organism and to the drug. Full details of drug doses are given in the protocols of the animal experiments.

EXAMPLE

TOXICITY TEST

COMPOUND II - p-hydroxybenzenesulphonamide

Soluble in water.

DOSE 20 mgs., 40 mgs., 60 mgs., and 80 mgs., each dissolved in 1 ml. sterile water and fed to 4 mice.

	Days after experiment							
	1	2	3	3	1	2	3
20 mgs. to each of 4 mice.	1.VF 2.VF 3.VF 4.VF	VF VF VF VF	VF VF VF VF	60mgs. to each of 4 mice.	1. X 2. X 3. VS[X] 4. S	VF	VF
40 mgs. to each of 4 mice.	1. S 2. VF 3. VF 4. VF	VF VF VF VF	VF VF VF VF	80mgs. to each of 4 mice.	1. X 2. X 3. X 4. X		

VF = Very Fit; S = Sick; VS = Very Sick; X = Death

Thus an approximate measure of the toxicity of this compound is 50 or 60 mgs. A safe dose would be from 20 to 30 mgs. and such was employed.

TABLE OF TOXICITIES OF COMPOUNDS

Compound Number	Name	Toxic dose for mouse of 20 mgs.
1	p-aminobenzenesulphonamide	80 mgs.
11	p-hydroxybenzenesulphonamide	50-60 mgs.
111	3-nitro-4-hydroxybenzenesulphonamide	100 mgs.
1V	3-amino-4-hydroxybenzenesulphonamide	80 mgs.
V	p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide	20 mgs.
V1	p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline	20 mgs.
V11	p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate	100 mgs.
V111	4.4'-dinitrodiphenylsulphide	100 mgs.
1X	4.4'-diaminodiphenylsulphide	60 mgs.
X	4.4'-diacetyldiaminodiphenylsulphide	100 mgs.
X1	3.5.dinitro-4-hydroxybenzenesulphonamide	60 mgs.
X11	Methylquinolylbenzenesulphonamide	NOT TESTED
X111	3-nitro-4-aminobenzenesulphonamide	70-80 mgs.
X1V	M. & B. 693 (2-sulphanilylamino-pipyridine)	100 mgs.
XV	1:4 di(p-acetylaminobenzenesulphonyl) piperazine	100 mgs.
XV1	sulphonamidobenzenediazonium chloride	20 mgs.
XV11	piperidine-1-piperidino-acridone-3-sulphonate	60 mgs.

TABLE OF TOXICITIES OF COMPOUNDS (Continued)

Compound Number	Name	Toxic dose for mouse of 20 mgs.
XVlll	4.4'-dimethyldiquinolyl-2-2' sulphide	NOT TESTED
XlX	1.4 di(p-aminobenzenesulphonyl) piperazine	80 mgs.
XX	Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate	100 mgs.
XXl	Ethyl-4-aminobenzenesulphonyl-piperazine carboxylate	80 mgs.
XXll	p-aminobenzenesulphonamide coupled with p-hydroxy-arsonic Acid	60 mgs.

ORGANISMS

The chief organisms used in this investigation were as follows:-

A. β -haemolytic streptococcus

B. Pneumococcus

C. Staphylococcus

D. Bacillus Aertrycke

The results obtained in these animal experiments are concisely summarised in Table 1. This table contains the essential results of a large number of individual experiments which are described in greater detail in pages 51 - 147.

Discussion of the results is best deferred to Section III (page 251) of the thesis as comparison can then be made with the results obtained from the in vitro tests.

TABLE 1

IN VIVO EXPERIMENTS

Therapeutic Activity for Mice

Compound Number	H. Streptococcus	Pneumococcus	Staphylococcus.	Bacillus Aertrycke
1	+++	+ to ±	± to 0	±
11	± to 0	± to 0	± to 0	0
111	++	0	0	0
1V	0	0	0	0
V	± to 0	0	0	0
V1	± to 0	± to 0	0	0
V11	± to 0	± to 0	0	0
V111	0	0	0	0
1X	± to 0	± to 0	0	0
X	0	± to 0	+	0
X1	±	-	± to 0	0
X11	0	0	-	-
X111	++ to +	-	-	0
X1V	+++	-	± to ±	0
XV	++	-	+ to ±	0
XV1	-	± to 0	0	-
XV11	0	± to 0	0	0
XV111	-	-	0	-
X1X	++ to +	-	± to 0	0
XX	0	-	0	-
XX1	0	-	0	-

KEY: +++ = 100 per cent recovery
++ = Approximately 75 per cent recovery
+ = " 30 " " "
± = Death delayed
0 = No effect
- = Not tested.

SECTION A

HAEMOLYTIC STREPTOCOCCUS

Strain	Source
A	Scarlet Fever
B	Mastoid
C	Tonsillitis
D	Carbuncle pus
E	Mastoid
F	Erysipelas
G	Pleural Fluid
H	Pus from scalp

SECTION A

/3 - HAEMOLYTIC STREPTOCOCCUS

ISOLATION OF STRAINS

Many strains of this organism were employed; they were isolated from tonsillitis, pneumonia, erysipelas, mastoid, scarlet fever, carbuncles and various minor infections.

The organisms were isolated from red blood agar culture when individual dew-drop colonies, showing true haemolysis, were picked on to serum agar slopes.

Haemolytic streptococcal cultures were sub-cultured once each week.

ESTIMATION OF VIRULENCE

Each new strain, after being isolated and tested for haemolytic activity, was immediately examined for its virulence on the mouse.

Mice of 20 - 25 grams were used in all experiments, and each mouse was weighed and the weight recorded.

Tenfold dilutions were made from an 18 hour broth culture, either in tubes of the broth medium or in 5 per cent mucin. With some strains in order to reduce their animal lethal dose for/

for mice it was necessary to suspend them on the surface of a mucous membrane. Miller and Castles (1938) found that hog's gastric mucin was a highly satisfactory medium in which to suspend the meningococcus for mouse inoculation, and this method has now been extended to many other organisms.

1 ml. of the bacterial dilution was used as the inoculum which was injected intraperitoneally into each mouse. The highest dilution killing within 24 hours 75 - 100 per cent of the animals injected was taken as a measure of the virulence of that particular strain.

All mice dying during the course of the experiments were examined post mortem. Unless mentioned otherwise, the organism originally injected was isolated from the heart blood of the dead animal.

ANIMAL EXPERIMENTS

A-HAEMOLYTIC STREPTOCOCCUS

COMPOUND 1 p-aminobenzenesulphonamide

DOSE- 20 mgs dissolved in 1 ml. water fed immediately after injection of culture and later as stated.

STRAIN- Haemolytic Streptococcus A 18 hour slope culture emulsified with saline and diluted 1 in 1,000 with animal mucin 1 ml. used as inoculum, injected intraperitoneally.

EXPERIMENT 1

		Days after Injection						
		1	2	3	4	5	6	7
Control mice	1	S	X					
1 ml. culture only	2	S	X					
	3	VF	VSx					
	4	VF	VSx					
Compound mice		VF	SS	X				
Immediately after culture 20mgs		VF	VF	VF	VF	VF	VF	VF
5 hours later 20mgs		VF	VF	VF	VF	VF	VF	VF
24 " " 20mgs								
48 " " 20 mgs		VF	VF	VF	VF	VF	VF	VF
72 " " 20mgs								

VF = Very Fit
S = Sick
SS = Slightly sick
VS = Very sick
X = Death

HAEMOLYTIC STREPTOCOCCUS A

COMPOUND 1 p-aminobenzenesulphonamide

DOSE- - 20 mgs fed immediately after culture, after 5 hours and daily for 3 - 4 days.

STRAIN- 18 hour culture diluted 1 in 1000 with mucin 1 ml. injected intraperitoneally.

EXPERIMENT 11		Days after Experiment				
		1	2	3	4	
Control Mice	1	X				
	2	VS	X			
	3	S	X			
	4	S	VS	X		
Compound Mice		VF	VF	VF	VF	
		VF	VF	VF	VF	
		VF	VF	VF	VF	survived indefinitely.
		VF	VF	VF	VF	

HAEMOLYTIC STREPTOCOCCUS STRAIN B - MASTOID

COMPOUND p-aminobenzenesulphonamide

DOSE - In water. 20 mgs per ml. given immediately after culture after 5 hours and daily for 3 - 4 days.

STRAIN - 18 hour culture diluted 1 in 1000. Dose of 1 ml. with mucin.

EXPERIMENT 111		Days after Injection							
		1	2	3	4	5	6	7	8
Control Mice	1	VS[X]							
1 ml. Strain B	2	VS[X]							
	3	S							
	4	S							
	5	VF	X						
	6	VF	X						
	7	VF	X						
	8	VF	X						
	9	VF	X						
	10	VF	VF	X					
Animals fed		VF	VS	X					
with compound		VF	S	S	X				
after culture		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF
		VF	VF	VF	VF	VF	VF	VF	VF

From these experimentes activity of p-aminobenzenesulphonamide against the haemolytic streptococcus shows almost 100 per cent recovery of the mice.

HAEMOLYTIC STREPTOCOCCUS

COMPOUND II p-hydroxybenzenesulphonamide

DOSE - 10 mgs.per ml. dissolved in water. Fed immediately after the injection of culture after 5 hours and if any survivors daily for 1 week.

STRAIN - Haemolytic Streptococcus A. 1 in 1,000 dilution in mucin of 18 hour culture. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	X						
	2	X						
	3	X						
	4	X						
Compound Mice	1	X						
	2	X						
	3	X						
	4	X						

EXPERIMENT II

COMPOUND II — Haemolytic Streptococcus C. 18 hour culture emulsified and diluted 1 in 1,000 with mucin,

DOSE - 1 ml. injected. in 10 mgs. fed immediately, after 5 hours and daily (if survivors).

	Days after Experiment			
	1	2	3	4
Controls	X			
1 ml. in 1 in	X			
1,000 culture	X			
	X			
Compound	X			
Start 5 hours	VS	X		
	S	X		
	S	S	X	

Thus Compound II has practically no chemotherapeutic effect on the haemolytic streptococcus. Perhaps it may slightly delay the death of the mice fed with it, as compared with the control mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND III — 3 nitro-4-hydroxybenzenesulphonamide dissolved in a weak solution of sodium bicarbonate.

DOSE - 20 mgs. in 1 ml. fed immediately after the culture after 4 hours, and daily as long as the mice survived.

STRAIN - Haemolytic Streptococcus B 18 hour culture emulsified and diluted 1 in 1,000 in mucin 1 ml.used as inoculum.

EXPERIMENT 1

	Days after Injection							
	1	2	3	4	5	6	7	8
Control mice culture only.	X							
	S	X						
	S	X						
	SS	S	X					
Compound fed daily.	VF	VF	VF	VF	VF	VF	VF	VF
	VF	VF	VF	VF	VF	VF	VF	VF
	VF	VF	VF	VF	VF	VF	VF	VF
	VF	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

EXPERIMENT II

COMPOUND III dissolved in Sodium Bicarbonate 20

DOSE - 20 mgs. in 1 ml. fed immediately after injection of organisms, after 5 hours and daily.

STRAIN - Haemolytic Streptococcus B. 18 hour culture emulsified and diluted 1 in 1,000 in mucin. 1 cc used as inoculum.

		Days after injection									
		1	2	3	4	5	6	7	8	9	10
Control Mice	1	VS[x]									
	2	VS[x]									
	3	S									
	4	S									
	5	VF	X								
	6	VF	X								
	7	VF	X								
	8	VF	X								
	9	VF	X								
	10	VF	S	X							
Com-	1	VF	X								
pound	2	VF	X								
Mice	3	VF	X								
	4	VF	S	VF	VF	VF	VF	X			
	5	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	6	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	7	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	8	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	9	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	10	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF

EXPERIMENT III

COMPOUND III

DOSE - 2^0 mgs. per 1 ml of compound dissolved in sodium bicarbonate fed immediately after culture injection, after 5 hours and daily for 7 - 10 days.

STRAIN - Haemolytic Streptococcus A (Scarlet fever) 1 cc of 1 in 1,000 dilution in mucin of 18 hour culture injected intraperitoneally.

		Days after Injection						
		1	2	3	4	5	6	7
Control	1	X						
Mice	2	S	VS	X				
	3	S	S	X				
	4	S	S	VS	X			
Compound	1	VF	VF	VF	VF	VF	VF	VF
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death.

From these experiments it was concluded that 3-nitro-4-hydroxybenzenesulphonamide has a definite effect ona fatal dose of the Haemolytic Streptococcus in mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND IV 3-Amino-4-hydroxybenzenesulphonamide

This compound was found to be insoluble in water, and thus, a suspension was made in 20 per cent gum acacia. As the toxicity was found to be low the dosage was taken as 20 milligrams per ml.

DOSE - Fed immediately after the culture and after 5 hours. Later daily if survivors.

STRAIN - Haemolytic Streptococcus D 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally to each mouse.

EXPERIMENT		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	VS	VS	X	
Compound Mice	1	VS	X		
	2	VS	X		
	3	VS	X		
	4	S	S	X	

S = Sick
VS = Very Sick
X = Death.

This compound was thus shown to have little or no effect as a chemotherapeutic agent against a haemolytic streptococcus infection of mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND V̄ p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide. Soluble in water.

DOSE - 10 mgs. per ml. in water given immediately after culture and after 5 hours and 24 hours

STRAIN - Haemolytic Streptococcus A. 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum.

		Days after Injection		
		1	2	3
Control Mice	1	S	X	
	2	S	X	
	3	S	X	
	4	S	X	
Compound Mice	1	S	X	
	2	S	X	
	3	S	S	X
	4	S	S	X

S = Sick
X = Death

This compound was thus shown to have little or no effect against a haemolytic streptococcus of mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND VI p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.
Very insoluble in water, and thus, suspended in gum acacia and water.

DOSE - 10 mgs in 1 ml water administered immediately after the culture, after 5 hours and after 24 hours.

STRAIN - Haemolytic Streptococcus D 18 culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1		Days after Injection			
		1	2	3	4
Control Mice	1	X			
Culture Only	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	SS	S	X	

SS = Slightly Sick
S = Sick
X = Death

EXPERIMENT II

COMPOUND VI

DOSE - 10 mgs. immediately after culture after 5 hours and later is survivors.

STRAIN - Haemolytic Streptococcus A 18 hour culture diluted 1 in 1,000. 1 ml. used as the inoculum and injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

This compound has been shown to have practically no effect against two different strains of the β Haemolytic Streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND VII p-aminobenzenesulphonamide coupled with 8-hydroxyquinolinemethosulphate. Slightly soluble in water.

DOSE - 10 mgs. in water fed immediately after the culture after 5 hours and later if any mice survived.

STRAIN - Haemolytic Streptococcus A 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death.

COMPOUND VII

EXPERIMENT II

DOSE - 10 mgs. in water after culture after 5 and 24 hours.

STRAIN - Haemolytic Streptococcus D 18 hour culture diluted 1 in 1000 in mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	SS	X		
	4	SS	S	X	

SS = Slightly Sick
S = Sick
X = Death

This compound has little or no chemotherapeutic effect against a virulent strain of the β-Haemolytic Streptococcus in a mouse infection.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND VIII 4.4'-dinitrodiphenylsulphide

Insoluble in water and thus suspended in 20 per cent gum acacia and water solution.

DOSE - 10 - 20 mgs. suspended in 1 ml. of liquid fed immediately after the injection of the culture, after 5 hours and after 24 hours.

STRAIN - Haemolytic Streptococcus A 18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	VS x		
	4	VF	VS x		
Compound Mice	1	VF	X		
	2	VF	X		
	3	VF	VSx		
	4	VF	VSx		

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

COMPOUND VIII

EXPERIMENT II

DOSE - 10 mgs. suspended in 1 ml. water fed immediately after culture and after 5 hours.

STRAIN - Haemolytic Streptococcus E 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

This compound has no chemotherapeutic effect against two strains of the β-Haemolytic Streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND IX - 4.4' diaminodiphenylsulphide dissolved in a weak solution of lactic acid and the solution neutralised with sodium bicarbonate. The sulphide is reprecipitated in a very fine form.

DOSE - 10 - 2^0 mgs. in 1 ml. of liquid fed immediately after the culture, after 5 hours, and after 24 hours.

STRAIN - Haemolytic Streptococcus A. 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as inoculum and injected intraperitoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	VS X		
	4	VF	VS X		
Compound Mice	1	VF	VF	X	
	2	VF	VF	X	
	3	VF	VF	X	
	4	VF	VF	X	

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

COMPOUND IX

EXPERIMENT II

DOSE - 10 mgs. in 1 ml. reprecipitated from dilute lactic acid. Fed immediately after culture and after 5 hours.

STRAIN - Haemolytic Streptococcus A 18 hour culture emulsified with saline and diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

From these experiments it is clear that this compound, 4.4'diaminodiphenylsulphide has no chemotherapeutic effect on haemolytic streptococcal infections in mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND X Diacetyldiaminodiphenylsulphide

DOSE - 10 - 20 mgs. fed in a 20 per cent suspension of gum acacia and water, immediately after the culture, after 5 and 24 hours.

STRAIN - Haemolytic Streptococcus D 18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	VS	VS	X	
Compound Mice	1	VS	X		
	2	S	X		
	3	S	X		
	4	VF	S	X	

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound has little or no effect on the haemolytic streptococcal septicaemia of the mouse.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XI 3.5 dinitro-4-hydroxybensenesulphonamide.

Soluble in Sodium Bicarbonate.

DOSE - 10 mgs. in 1 ml. dissolved in dilute $NaHCO_3$ fed immediately after the culture injection, after 5 hours, 24 hours and daily as long as the mice survived.

STRAIN - Haemolytic Streptococcus A 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	S	VS	X				
	2	S	S	X				
	3	S	S	S	S	S	X	
Compound Mice	1	VF	VF	X				
	2	VF	VF	VF	SS	S	X	
	3	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound has a slight death retarding effect on a fatal dose of the haemolytic streptococcus in mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XII — Methylquinolylbenzenesulphonamide

Insoluble in water. A dilute solution in lactic acid employed.

DOSE - 10 mgs. per ml. employed as test dose administered immediately after culture after 5 and 24 hours.

STRAIN - Haemolytic Streptococcus F

18 hour culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	VS[X]			
	3	VS[X]			
	4	VS[X]			
Compound Mice	1	X			
	2	X			
	3	VS[X]			
	4	VS[X]			

VS = Very Sick
X = Death

This compound has no chemotherapeutic effect on a haemolytic streptococcal septicaemia in mice.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XIII 3-nitro-4-aminobenzenesulphonamide insoluble in water, and therefore fed in a 20 per cent suspension of gum acacia and water.

DOSE - 30 mgs. in 1 ml. liquid fed immediately after the culture, after 5 hours, 24 hours, 30 hours and daily as long as the mice survived.

STRAIN - Haemolytic Streptococcus G 18 hour culture dilutedlin 1,000 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	X				
	3	X				
	4	X				
Compound Mice	1	X				
	2	X				
	3	X				
	4	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

Examination at post-mortem showed that all the deaths were due to the haemolytic streptococcus.

COMPOUND XIII 3-nitro-4-aminobenzenesulphonamide

EXPERIMENT II

DOSE - as in Experiment 1

STRAIN - Haemolytic Streptococcus H 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum.

		Days after Injection					
		1	2	3	4	5	6
Control Mice	1	X					
	2	X					
	3	X					
	4	X					
Compound Mice	1	VF	VF	VF	VF	VF	VF
	2	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

One of the four compound mice died after 15 days, another after 22 days. The post-mortem examination revealed haemolytic streptococci in the heart blood of both mice. The two surviving mice were killed after 1 month. At post-mortem examination both seemed perfectly healthy and it was not possible to isolate haemolytic streptococci from the heart blood.

COMPOUND XIII 3-nitro-4-aminobenzenesulphonamide

EXPERIMENT III

DOSE - 30 mgs. in 1 ml. liquid twice daily for 2 days and once daily for 1 week.

STRAIN - Haemolytic Streptococcus G 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	X						
	2	X						
	3	VS	X					
	4	VS	X					
Compound Mice	1	VF	VF	VF	VF	VF	VS (killed) *	
	2	VF	VF	VF	VF	VF	VF	
	3	VF	VF	VF	VF	VF	VF	
	4	VF	VF	VF	VF	VF	VF	

* Killed as suffering from severe paralysis of the hind legs.

The other three mice were kept for one month at the end of which time they were found to be perfectly healthy when killed and examined at post-mortem.

This compound, 3-nitro-4-aminobenzene-sulphonamide, has a definite curative action on the haemolytic streptococcal septicaemia of the mouse.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XIV — 2-sulphanilylaminopyridine or M. & B. 693.

This compound was found to be very slightly soluble in water. It had, however, to be fed to the mice in a suspension in 20 per cent gum acacia and water.

DOSE - 20 mgs. twice daily for two days and then daily for 1 week.

STRAIN - Haemolytic Streptococcus G 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum which was injected intra-peritoneally.

EXPERIMENT 1

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	X						
	2	X						
	3	X						
	4	X						
Compound	1	VF	VF	VF	VF	VF	VF	VF
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

COMPOUND XIV 2-sulphanilylaminopyridine

EXPERIMENT 11

DOSE - As in Experiment 1

STRAIN - Haemolytic Streptococcus H 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

		1	2	3	4	5	6	7
Control Mice	1	X						
	2	X						
	3	X						
	4	X						
Compound	1	VF	VF	VF	VF	*X		
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

* Death from haemolytic streptococcal infection.

2-sulphanilylaminopyridine is very active against mouse haemolytic streptococcal septicaemia. Its activity may be compared to that of p-aminobenzenesulphonamide.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XV 1:4 di(p-acetylaminobenzenesulphonyl) piperazine.

Insoluble in water and was administered to the mice in the form of a suspension with 20 per cent gum acacia.and water.

DOSE - 30 mgs. suspended in 1 ml. of liquid fed immediately after the culture, after 5 hours, 24 hours, and daily for 1 week.

STRAIN - Haemolytic Streptococcus G. 18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum.

EXPERIMENT 1		Days after Injection						
Control Mice		1	2	3	4	5	6	7
	1	X						
	2	X						
	3	X						
	4	X						
Compound	1	VF	VF	VF	VF	VF	VF	VF
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

One mouse died after 14 days from a chronic haemolytic streptococcal infection.

COMPOUND XV 1:4di(p-acetylaminobenzenesulphonyl) piperazine.

DOSE - as in Experiment 1.

STRAIN - Haemolytic Streptococcus H 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

EXPERIMENT II

		Days after Injection						
Control Mice		1	2	3	4	5	6	7
	1	X						
	2	X						
	3	X						
	4	X						
Compound	1	VF	VF	VF	VF	VF	X	
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

At post-mortem examination the mouse dying on the sixth day was found to have haemolytic streptococci in its heart blood.

This compound, 1:4 di(p-acetylaminobenzenesulphonyl) piperazine, certainly has some effect against mouse haemolytic streptococcal septicaemia though it may only be a death delaying effect.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XVII Piperidine-1-piperidino-acridone-3-sulphonate.

DOSE - Very soluble in water. 20 mgs. in 1 ml. fed immediately after the culture injection and after 5 and 24 hours.

STRAIN - Haemolytic Streptococcus H. 18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. used as the inoculum and injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Compound XVII has no effect on a mouse haemolytic streptococcal septicaemia.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XIX — 1:4 di(p-aminobenzenesulphonyl) piperazine.

DOSE - Suspended in a 20 per cent gum acacia and water solution. 20 mgs. in 1 ml. fed immediately after the culture injection, after 5 hours, 18 hours, 24 hours and daily for 1 week.

STRAIN - Haemolytic Streptococcus H. 18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection									
		1	2	3	4	5	6	7	8	9	10
Control Mice	1	X									
	2	X									
	3	X									
	4	X									
Compound Mice	1	VF	VF	X							
	2	VF	VF	VF	VF	X					
	3	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

COMPOUND XIX

EXPERIMENT II

DOSE - as in Experiment 1.

STRAIN - as in Experiment 1.

		Days after Injection									
		1	2	3	4	5	6	7	8	9	10
Control	1	X									
Mice	2	X									
	3	X									
	4	X									
Compound	1	X									
Mice	2	VF	VF	VF	VF	X					
	3	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

Compound XIX has a definite antistreptococcal action in a mouse infection.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate.

DOSE - Insoluble in water. Fed in a 20 per cent gum acacia water solution. 20 mgs. in 1 ml. immediately after the culture and after 5 hours.

STRAIN - Haemolytic Streptococcus H. 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

Compound XX has no effect on a mouse haemolytic streptococcal septicaemia.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XXI — Ethyl-4-aminobenzenesulphonyl-piperazine-1-carboxylate.

DOSE - Insoluble in water and thus fed in a gum acacia water suspension. 20 mgs. in 1 ml. fed immediately after the culture and after 5 hours.

STRAIN - Haemolytic Streptococcus H. 18 hour culture diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Compound XXI has no curative effect on a mouse haemolytic streptococcal septicaemia.

SECTION II

Part 1 - In Vivo Experiments

B. PNEUMOCOCCUS

ISOLATION OF STRAINS

The strains used in this investigation were obtained from various sources. One millilitre of the material was injected intraperitoneally into a mouse of approximately 20 grams weight . If a sufficiently great number of organisms were present, the mouse died within 18 - 24 hours. At the post-mortem examination, the peritoneal cavity showed the typical picture of a pneumococcal peritonitis. The turbid exudate, which on microscopic examination showed numerous capsulated coccal pairs, was used to type the organism.

The cultures were isolated in red blood agar, from which media they were subcultured on to serum agar slope tubes. The various strains were stored on these tubes, and they were subcultured once every week.

B. PNEUMOCOCCUS		SOURCE
Strain X	Type II	Pleural Effusion
Strain Y	Type II	C.S.F.
Strain Z	Type II	Sputum
Strain P	Group IV	Sputum
Strain Q	Type I	Blood Stream
Strain R	Group IV	Sputum

VIRULENCE TESTS

Virulence tests were carried out on the various strains of the pneumococcus. Dilutions were made in sterile saline and 1 ml. was used as the inoculum, and injected intraperitoneally.

With each strain, the highest dilution killing 100 per cent of the mice within 18 - 24 hours, was used as a measure of virulence.

B. PNEUMOCOCCUS

COMPOUND I p-aminobenzenesulphonamide

DOSE - 20 mgs. used as the test dose. Fed to the mice immediately after the injection of the culture, after 5 hours, and twice daily as long as the mice survived.

STRAIN - Pneumococcus Z (Type II) 18 hour culture on serum agar emulsified and diluted 1 in 1,000 with sterile saline. 1 ml. used as inoculum and injected intra-peritoneally.

EXPERIMENT 1

		Days after Injection				
		1	2	3	4	5
Control Mice	1	S	X			
	2	S	X			
	3	VF	X			
	4	VF	X			
Compound I Mice	1	VF	VF	S	X	
	2	VF	VF	VF	VS	
	3	VF	VF	VF	VF	X
	4	VF	VF	VF	VF	X

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

B. PNEUMOCOCCUS

COMPOUND I p-aminobenzenesulphonamide

EXPERIMENT II

DOSE - As in experiment 1.

STRAIN - Pneumococcus (Type II) Z. diluted as in Experiment 1.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	X		
	4	VF	X		
Compoubd Mice	1	VF	VF	X	
	2	VF	VF	X	
	3	VF	VF	X	
	4	VF	VF	X	

VF = Very Fit
S = Sick
X = Death

These experiments show that p-aminobenzene-sulphonamide has some slight effect on a mouse pneumococcal peritonitis. It delays death by approximately 24 hours, but it does not cure the animals.

B. PNEUMOCOCCUS

COMPOUND II — p-hydroxybenzenesulphonamide

DOSE - Soluble in water. Solution containing 20 mgs. per ml. made up. 1 ml. fed immediately after the culture injection, after 5 hours and twice daily as long as the mice survived.

STRAIN - Pneumococcus Y (Type II) 18 hour culture diluted 1 in 1,000 with sterile saline. 1 ml. injected intraperitoneally.

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	X						
	2	S	X					
	3	S	X					
	4	SS	SS	SS	X			
Compound	1	VF	X					
Mice	2	VF	VF	VF	X			
	3	VF	VF	VF	S	X		
	4	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound, p-hydroxybenzenesulphonamide, has a slight death delaying effect on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DOSE - Insoluble in water but soluble in sodium bicarbonate. Fed in the form of a weak solution with sodium bicarbonate. 20 mgs. per ml. used as the test dose fed immediately after the culture injection, after 5 hours, 24 hours and daily.

STRAIN - Pneumococcus P (Group IV)
18 hour slope culture emulsified with saline and diluted 1 in 1,000. 1 ml. used as the inoculum and injected intraperitoneally.

EXPERIMENT 1

		Days after Injection				
		1	2	3	4	5
Control Mice	1	VF	S	X		
	2	VF	S	X		
	3	VF	S	X		
	4	VF	SS	X		
	5	VF	SS	VS	X	
	6	VF	VF	SS	S	X
Compound Mice	1	VF	S	X		
	2	VF	SS	X		
	3	VF	VF	VS	X	
	4	VF	VF	S	X	
	5	VF	VF	S	X	
	6	VF	VF	S	S	X

COMPOUND III 3-nitro-4-hydroxybenzenesulphonamide.

EXPERIMENT II

DOSE- as in Experiment 1

STRAIN - Pneumococcus X

18 hour culture diluted 1 in 1,000 with saline. 1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	SS	X			
	2	SS	S	X		
	3	SS	S	SS	X	
Compound Mice	1	SS	VF	VF	X	
	2	SS	VF	S	X	
	3	SS	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound 3-nitro-4-hydroxybenzenesulphonamide has little effect on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

DOSE - 20 mgs. in 1 ml. of a 20 per cent gum acacia and water suspension fed immediately after the injection of culture, after 5 hours and 24 hours.

STRAIN - Pneumococcus X
18 hour culture emulsified and diluted 1 in 1,000 with saline. 1 ml. used as the inoculum and injected intraperitoneally.

EXPERIMENT 1

		Days after Injection				
		1	2	3	4	5
Control Mice	1	SS	S	X		
	2	VF	S	S	X	
	3	VF	S	VF	VF	VF
Compound Mice	1	VS	X			
	2	VS	VS	X		
	3	S	S	S	S	X

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

EXPERIMENT II

DOSE - Pneumococcus X
18 hour culture emulsified and diluted 1 in 1,000 with saline.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	SS	S	X	
	3	SS	SS	X	
Compound Mice	1	SS	S	X	
	2	SS	VF	S	X
	3	SS	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

3-amino4-4hydroxybenzenesulphonamide has little or no effect on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND V — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide.

DOSE - This compound is slightly soluble in water. 5 mgs. per millilitre was fed immediately after the culture, after 5 hours and 24 hours.

STRAIN - Pneumococcus Z
18 hour culture diluted 1 in 100 with saline. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	X		
	4	VF	X		
Compound Mice	1	VF	X		
	2	VF	X		
	3	VF	VS[X]		
	4	VF	S	X	

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

Compound V has little or no effect on a mouse pneumococcal peritonitis.

COMPOUND VI — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DOSE - Insoluble in water and thus suspended in a 20 per cent solution with gum acacia and water. 5 mgs. fed immediately after the culture after 5 hours and 24 hours.

STRAIN - Pneumococcus Z
18 hour culture emulsified and diluted 1 in 100 with sterile saline. 1 ml. injected intra-peritoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	VF	X		
	2	VF	X		
	3	VF	VS	X	
	4	VF	VS	X	
Compound Mice	1	VF	X		
	2	VF	VS	X	
	3	VF	S	X	
	4	VF	VF	X	

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

COMPOUND VI p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.
EXPERIMENT 11

DOSE - as in Experiment 1.

STRAIN - Pneumococcus Z 18 hour culture emulsified, and diluted 1 in 100 with saline. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	X		
	4	VF	X		
Compound Mice	1	VF	X		
	2	VF	X		
	3	VF	X		
	4	VF	X		

VF = Very Fit
S = Sick
X = Death

Compound VI, p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline, has no effect on mouse pneumococcal peritonitis.

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinolinemethosulphate.

DOSE - This compound was found to be slightly soluble in water. The dose fed to the mice was 5 mgs. in 1 ml. of water immediately after the culture, after 5 hours and after 24 hours.

STRAIN - Pneumococcus Z
18 hour culture emulsified and diluted 1 in 100 with sterile saline. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	VF	X		
	2	VF	X		
	3	VF	X		
	4	VF	X		
Compound Mice	1	VF	S^X		
	2	VF	SS	X	
	3	VF	VF	X	
	4	VF	VF	X	

VF = Very Fit
SS = Slightly Sick
S = Sick
X = Death

COMPOUND VII

EXPERIMENT II

DOSE - As in Experiment 1.

STRAIN - Pneumococcus strain P.
18 hour culture emulsified and diluted 1 in 10 with saline.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	S	X		
Compound Mice	1	S	X		
	2	SS	S	X	
	3	VF	S	X	
	4	VF	S	X	

VF = Very Fit
SS = Slightly Sick
S = Sick
X = Death

This compound, p-aminobenzenesulphonamide coupled with 8-hydroxyquinolinemethosulphate has little or no effect on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND VIII — 4.4' dinitrodiphenylsulphide

DOSE - As this compound was found to be insoluble in water it was fed to the mice in the form of a 20 per cent suspension with gum acacia and water. 20 mgs. were fed immediately after the culture, after 5 hours and 24 hours.

STRAIN - Pneumococcus X
18 hours culture emulsified and diluted 1 in 100 with sterile saline. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VF	X		
	2	VF	X		
	3	VF	X		
	4	VF	X		
	5	VF	X		
	6	VF	X		
Compound Mice	1	VF	X		
	2	VF	X		
	3	VF	X		
	4	VF	VS	X	
	5	VF	VS	X	
	6	VF	VS	X	

VF= Very fit
VS= Very sick
X= Death

This compound, 4.4'dinitrodiphenylsulphide has no

B. PNEUMOCOCCUS

COMPOUND IX — Diaminodiphenylsulphide.

DOSE - This compound was found to be slightly soluble in dilute acid. A dilute solution was made with lactic acid. 10 mgs. in 1 ml. were fed immediately after the injection of the culture, after 5 hours and 24 hours.

STRAIN - Pneumococcus Z
18 hour culture emulsified and diluted 1 in 100. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	S	X		
	3	VS	X		
	4	VF	X		
Compound Mice	1	VF	X		
	2	VF	X		
	3	VF	VS	X	
	4	VF	S	X	

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

COMPOUND IX

EXPERIMENT II

DOSE - As in Experiment 1.

STRAIN - Pneumococcus Z.
18 hour culture diluted 1 in 100 with saline. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VF	X		
	2	VF	X		
	3	VF	X		
	4	VF	X		
Compound Mice	1	VF	X		
	2	VF	S	X	
	3	VF	S	X	
	4	VF	VF	X	

VF = Very Fit
S = Sick
X = Death

This compound has little or no effect on mouse pneumococcal peritonitis. It may delay death by some hours.

B. PNEUMOCOCCUS

COMPOUND X — Diacetyldiaminodiphenylsulphide.

DOSE - Insoluble in water and thus fed in a 20 per cent gum acacia and water suspension. 20 mgs. fed immediately after the culture injection after 5 hours and after 24 hours.

STRAIN - Pneumococcus Y
18 hour culture emulsified and diluted 1 in 100 with saline. 1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	S	X			
	3	S	X			
	4	SS	S	S	X	
Compound Mice	1	X				
	2	S	X			
	3	VF	S	VS	X	
	4	VF	VF	VF	VF	

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound, diacetyldiaminodiphenylsulphide may have a slight death delaying action on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND XII Methylquinolylbenzenesulphonamide

DOSE - Dilute solution made up with lactic acid. 10 mgs. fed in 1 ml. immediately after the culture injection, after 5 hours and after 24 hours.

STRAIN - Pneumococcus Y
18 hour culture emulsified and diluted 1 in 100 with saline.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	VS[X]			
	3	VS[X]			
	4	S	VF	VF	VF
Compound Mice	1	X			
	2	X			
	3	VS[X]			
	4	SS	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

This compound, methylquinolylbenzenesulphonamide has no effect on mouse pneumococcal peritonitis.

B. PNEUMOCOCCUS

COMPOUND XVI Sulphonamidobenzenediazoniumchloride

DOSE - This compound was found to be very toxic and thus 10 mgs. was taken as the maximum dose. As this compound is very unstable, the solution of it was prepared immediately before the mice were injected. 1 ml. equivalent to 5 - 10 mgs. was fed after the culture, after 5 hours and 24 hours and daily if the mice survived.

STRAIN - Pneumococcus Z.
18 hour culture emulsified and diluted 1 in 100 with saline.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	SS	VS[x]		
	4	SS	VS[x]		
Compound Mice	1	VF	VF	X	
	2	VF	VF	X[F]	
	3	VF	VF	X	
	4	VF	VF	X	

VF= Very Fit
SS= Slightly Sick (Sick
S=Sick
X=Death

Compound XVI , sulphonamidobenzenediazoniumchloride may have a slight death delaying effect on mouse pneumococcal peritonitis.

COMPOUND XVII Piperidine-1-piperidino-acridone-3-sulphonate.

DOSE - This compound was found to be very soluble in water and the toxicity was low. 20 mgs. were fed in 1 ml. of water immediately after the injection of the culture, after 5 houfs and 24 hours.

STRAIN - Pneumococcus Z
18 hour culture diluted 1 in 100.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	VF	VSx		
	4	VF	VSx		
Compound Mice	1	S	VSx		
	2	S	VSx		
	3	VF	S	X	
	4	VF	S	X	

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

Compound XVII has little or no effect on mouse pneumococcal septciaemia.

SECTION II

Part 1 - In Vivo Experiments

C. STAPHYLOCOCCUS

ISOLATION OF CULTURES

The material obtained was plated on red blood agar and incubated for 24 hours. The typical aureus colonies were then examined microscopically, and picked on to agar slopes. The cultures were stored on agar slopes and were subcultured once every week.

VIRULENCE TESTS

18 hour slope cultures of the isolated organisms were emulsified with saline and diluted 1 in 10 and 1 in 50 with 5 per cent animal mucin. If the strain was suitable for use in these experiments, that is, if the organisms killed injected mice within 24 - 48 hours, a virulence test was repeated using ten-fold dilutions and the exact dose killing all mice within 24 hours was ascertained.

SECTION C - STAPHYLOCOCCUS

SOURCE OF CULTURES

Strain	M	source	Osteomyelitis
Strain	N	source	Breast Boil
Strain	K	source	Heart Blood

C. STAPHYLOCOCCUS

COMPOUND 1 p-aminobenzenesulphonamide

DOSE - 20 mgs. dissolved in 1 ml. of water fed immediately after the culture injection, after 5 hours, and if any mice survived, after 24 hours.

STRAIN - Staphylococcus N 18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	VSx		
	3	VS	X	
	4	VS	X	

VS = Very Sick
X = Death

COMPOUND I p-aminobenzenesulphonamide

EXPERIMENT II

DOSE - As in Experiment 1.

STRAIN - Staphylococcus N
18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection									
		1	2	3	4	5	6	7	8	9	10
Control	1	X									
Mice	2	S	X								
	3	S	X								
	4	SS	X								
Compound	1	S	S	X							
Mice	2	S	SS	SS	SS	VF	X				
	3	SS	VF	VF	VF	VF	VF	VF	VF	VF	X
	4	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
X = Death

From these experiments it may be concluded that p-aminobenzenesulphonamide has a slight death delaying action on mouse staphylococcal septicaemia. However, as at post-mortem examination the organisms are found in the heart blood and, if the animal has lived for some time, numerous staphylococcal abscesses are found in the spleen and liver.

C. STAPHYLOCOCCUS

COMPOUND II p-hydroxybenzenesulphonamide.

DOSE - As this compound is soluble in water it was fed in a 0.1 per cent solution. 1 ml. equivalent to 10 mgs. was fed immediately after the culture after 5 hours and after 24 hours. If the mice survived daily doses were continued.

STRAIN - Staphylococcus N.
18 hour culture emulsified and diluted 1 in 100. 1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	S	X			
	3	SS	X			
	4	SS	X			
Compound Mice	1	X				
	2	S	X			
	3	S	SS	X		
	4	S	VF	SS	SS	X

V = Very Fit
SS = Slightly Sick
S = Sick
X = Death.

p-hydroxybenzene may have a slight death delaying action on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DOSE - Dissolved in sodium bicarbonate 20 mgs. in 1 ml. fed immediately after the culture injection, after 5 hours and after 24 hours.

STRAIN - Staphylococcus M. 18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

This compound 3-nitro-4-hydroxybenzenesulphonamide has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND IV — 3-amino-4-hydroxybenzenesulphonamide.

DOSE - 20 mgs. per ml. suspended in 20 per cent gum acacia and water solution. 20 mgs. fed immediately after the culture and after 5 hours.

STRAIN - Staphylococcus N
18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	VS	X	
	2	VS	X	
	3	VS	X	
	4	VS	X	
Compound Mice	1	S	X	
	2	S	X	
	3	S	X	
	4	S	X	

S = Sick
VS = Sick
X = Death

This compound, 3-amino-4-hydroxybenzene - sulphonamide has no effect on mouse staphylococcal setpicaemia.

C. STAPHYLOCOCCUS

COMPOUND IV — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide.

DOSE - Dissolved in water. 10 mgs. fed in 1 ml. immediately after the culture and after 5 hours.

STRAIN - Staphylococcus M

18 hour culture emulsified and diluted 1 in 50 with mucin 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	S	X		
	4	S	X		
Compound Mice	1	S	X		
	2	S	X		
	3	S	X		
	4	S	X		

S = Sick
X = Death

Compound V has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND VI — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DOSE - 10 mgs. in 1 ml. fed in a 20 per cent gum acacia and water suspension immediately after the culture injection, after 5 hours and 24 hours.

STRAIN - Staphylococcus M

18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VS	X		
	2	VS	X		
	3	VS	X		
	4	VS	X		
Compound Mice	1	VS	X		
	2	VS	X		
	3	S	VS[X]		
	4	S	VS	X	

S = Sick
VS = Very Sick
X = Death

This compound has little or no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate.

DOSE - Slightly soluble in water and thus fed in a dose of 10 mgs. per ml. immediately after the culture injection, after 5 hours and 24 hours.

STRAIN - Staphylococcus M.
18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

This compound has no effect on mouse staphylococcal setpicaemia.

C. STAPHYLOCOCCUS

COMPOUND VIII Dinitrophenylsulphide

DOSE - Suspension prepared with a 20 per cent solution of gum acacia and water. 20 mgs. per ml. and 1 ml. fed immediately after the culture injection, after 5 hours and 24 hours.

STRAIN - Staphylococcus N.
18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VS	X		
	2	VS	X		
	3	VS	X		
	4	VS	X		
Compound Mice	1	X			
	2	X			
	3	VS	X		
	4	VS	X		

VS = Very Sick
X = Death

This compound has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND IX Diaminodiphenylsulphide

DOSE - A weak solution of this compound was made with lactic acid. 10 mgs. fed in 1 ml. immediately after the culture injection, after 5 hours and after 24 hours.

STRAIN - Staphylococcus N.
18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	S	X		
	2	S	X		
	3	S	X		
	4	S	X		
Compound Mice	1	S	X		
	2	S	X		
	3	S	X		
	4	S	X		

S = Sick
X = Death

Diaminophenylsulphide has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND X — Diacetyldiaminodiphenylsulphide

DOSE - Insoluble in water and thus fed in the form in the form of a gum acacia and water suspension. 20 mgs. in 1 ml. fed immediately after the culture injection, after 5 hours and after 24 hours.

STRAIN - Staphylococcus N
18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection										
		1	2	3	4	5	6	7	8	9	10	11
Control Mice	1	X										
	2	S	X									
	3	S	X									
	4	SS	X									
Compound Mice	1	X										
	2	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF	VF

S = Sick
SS = Slightly Sick
VF = Very Fit
X = Death

EXPERIMENT 1 (Continued)

The three treated mice were killed at the end of one month. At the post-mortem examination, one was found to have a staphylococcal abscess in the liver and a staphylococcus culture was isolated from the heart blood. The two remaining mice were found to be perfectly healthy at post-mortem examination. It was not possible to isolate a staphylococcus culture either from the heart blood or from the organs.

COMPOUND X Diacetyldiaminodiphenylsulphide.

EXPERIMENT II

DOSE - As in Experiment 1

STRAIN - As in Experiment 1.

		Days after Injection					
		1	2	3	4	5	6
Control Mice	1	X					
	2	X					
	3	X					
———	4	X					
	5	X					
	6	X					
Compound Mice	1	X					
	2	VS	X				
	3	S	X				
	4	S	VS	S	S	X	
	5	VF	S	VF	VF	VF	X
	6	VF	VF	VF	VF	VF	VF

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

Diacetyldiaminodiphenylsulphide has a death delaying effect on mouse staphylococcal septicaemia. In some cases it may prevent death.

C. STAPHYLOCOCCUS

COMPOUND X1 3-5-dinitro-4-hydroxybenzene sulphonamide.

DOSE - Dissolved in sodium bicarbonate 10 mgs. in 1 ml. fed immediately after the culture injection, after 5 and 24 hours.

STRAIN - Staphylococcus M 18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VS	X		
	2	VS	X		
	3	VS	X		
	4	VS	X		
Compound Mice	1	VS	X		
	2	VS	X		
	3	VS	X		
	4	S	VF	VF	VF

VF = Very Fit
S = Sick
VS = Very Sick
X = Death

3.5 dinitro-4-hydroxybenzenesulphonamide may have a slight death delaying action on mouse staphylococcal septicaemia.

c. STAPHYLOCOCCUS

COMPOUND XIV 2-sulphanilylaminopyridine

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. fed immediately after the culture injection, after 5 hours, 24 hours and daily.

STRAIN - Staphylococcus K
18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

Days after Injection

Days after Injection

		1	2	3	4	5	6
Control Mice	1	X					
	2	X					
	3	VS	VS	S	S	VF	VF
	4	SS	VF	VF	VF	VF	VF
Compound Mice	1	S	VF	VF	VF	VF	VF
	2	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF

VF = Very Fit
SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

C. STAPHYLOCOCCUS

COMPOUND XV 1:4 di(p-acetylaminobenzenesulphonyl) piperazine.

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension in a dose of 20 mgs. in 1 ml. immediately after the culture after 5 hours and after 24 hours.

STRAIN - Staphylococcus K 18 hour cultured emsulsified and diluted 1 in 50 with mucin.

EXPERIMENT 1

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	S	X	
	3	S	X	
	4	VS	X	

COMPOUND XV

EXPERIMENT II.

DOSE - As in Experiment 1. Surviving mice fed daily with 20 mgs.

STRAIN - Staphylococcus K 18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection						
		1	2	3	4	5	6	7
Control Mice	1	X						
	2	X						
	3	VS	VS	VS	X			
	4	S	SS	VF	VF	VF	VF	VF
Compound	1	VF	VF	VF	VF	VF	VF	VF
Mice	2	VF	VF	VF	VF	VF	VF	VF
	3	VF	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF	VF

VF= Very Fit
SS= Slightly Sick
S= Sick
VS= Very Sick
X=-Death

1:4 di(p-acetylaminobenzenesulphonyl piperazine has a definite death delaying effect on a mouse staphylococcal infection.

C. STAPHYLOCOCCUS

COMPOUND XVI Sulphonamidobenzenediazonium chloride.

DOSE - 1 ml. of a solution which contained 10 mgs. per ml. was fed immediately after the culture injection

STRAIN - Staphylococcus N
18 hour culture emulsified and diluted 1 in 50 with mucin 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Sulphonamidobenzenediazonium chloride has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND XVII — Piperidine-1-piperidino-acridone-3-sulphonate.

DOSE - Very soluble in water. 10 mgs. in 1 ml. solution fed immediately after the culture injection, after 5 hours and 24 hours.

STRAIN - Staphylococcus N. 18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. Injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death.

This compound has no effect on mouse streptococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND VXIII — 4:4'-dimethyldiquinolyl-2:2'-sulphide.

DOSE - Insoluble in water and thus fed in a gum acacia water solution. 10 mgs. fed immediately after the culture injection, after 5 hours and 24 hours. Daily as long as the mice survived.

STRAIN - Staphylococcus K

78 hours emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection					
		1	2	3	4	5	6
Control Mice	1	X					
	2	X					
	3	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF
Compound Mice	1	X					
	2	VF	VF	X			
	3	VF	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF	VF

VF = Very Fit
X = Death

Remaining mice were killed after 10 days and examined at post-mortem. Staphylococci were isolated from the heart blood in each case. This compound has no effect on mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND XIX 1:4-di(p-aminobenzenesulphonyl) piperazine.

DOSE - Insoluble in water. Fed in a gum acacia and water suspension in a dose of 20 mgs. in 1 ml. immediately after the culture injection and after 5 hours.

STRAIN - Staphylococcus K.
18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	VS	X	
	4	VS	X	

VS = Very Sick
X = Death

1:4-di(p-aminobenzenesulphonyl)piperazine has littleor no effect on a mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension in a dose of 20 mgs. per ml. immediately after the culture injection and after 5 hours.

STRAIN - Staphylococcus K. 18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Compound XX has no effect on a mouse staphylococcal septicaemia.

C. STAPHYLOCOCCUS

COMPOUND XXI — Ethyl-4-aminobenzenesulphonyl piperazine-1-carboxylate.

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. in 1 ml. fed immediately after the culture and after 5 hours.

STRAIN - Staphylococcus K.
18 hour culture emulsified and diluted 1 in 50 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Compound XXI has no effect on a mouse staphylococcal infection.

SECTION II

Part 1 - In Vivo Experiments

D. BACILLUS AERTRYCKE

ISOLATION OF CULTURES

Two cultures only were employed:

1. BACILLUS AERTRYCKE U

   was obtained from the Bacteriology Department, Edinburgh University.

11. BACILLUS AERTRYCKE V

   was isolated from the spleen of a mouse.

Both cultures gave positive agglutination tests with specific Aertrycke serum.

The cultures were stored in ordinary agar slopes and were sub-cultured once every week.

VIRULENCE

Virulence tests were carried out frequently with both cultures. Ten-fold dilutions were made with sterile saline or with 5 per cent mucin, and the highest dilution killing 100 per cent of the injected mice was taken as a measure of the virulence of that strain.

D. BACILLUS AERTRYCKE

COMPOUND 1 p-aminobenzenesulphonamide

DOSE - 20 mgs. in 1 ml. water fed immediately after the culture injection after 5 hours and 24 hours.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000. 1 ml. injected intraperitoneally.

EXPERIMENT 1

		Days after Injection		
		1	2	3
Control Mice	1	VS[X]		
	2	VS[X]		
	3	X		
	4	X		
Compound Mice	1	X		
	2	S	X	
	3	SS	SS	X
	4	SS	SS	X

SS = Slightly Sick
S = Sick
VS = Very Sick
X = Death

p-aminobenzenesulphonamide has a slight death delaying effect on a bacillus aertrycke infection of mice.

D. BACILLUS AERTRYCKE

COMPOUND I p-aminobenzenesulphonamide

EXPERIMENT 11

DOSE - As in Experiment 1. Feeding was continued twice daily if the mice survived.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 1,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	X				
	3	X				
	4	X				
Compound Mice	1	VF	X			
	2	VF	S	X		
	3	VF	VF	VF	S	X
	4	VF	VF	VF	SS	VF

VF = Very Fit
S = Sick
SS = Slightly Sick
X = Death

p-aminobenzenesulphonamide has a slight death delaying effect on a Bacillus Aertrycke infection of mice.

D. BACILLUS AERTRYCKE

COMPOUND II — p-hydroxybenzenesulphonamide

DOSE - Soluble in water. 10 mgs. in 1 ml. fed immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 1,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

p-hydroxybenzenesulphonamide has no effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide

DOSE - 20 mgs. per ml. fed in a sodium bicarbonate solution immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 1,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

3-nitro-4-hydroxybenzenesulphonamide has no effect ona mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

DOSE - Suspension in gum acacia and water. 10 mgs. of the compound fed immediately after the culture and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 1,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	X				
	3	X				
	4	X				
Compound Mice	1	VS[X]				
	2	X				
	3	X				
T	4	X				

VS = Very Sick
X = Death

This compound, 3-amino-4-hydroxybenzene-sulphonamide has no effect on a Bacillus Aertrycke infection of mice.

D. BACILLUS AERTRYCKE

COMPOUND V — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide

DOSE - 10^0 mgs. dissolved in water fed immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	VS^X		
	2	VS^X		
	3	X		
	4	X		

VS = Very Sick
X = Death

Compound V has no curative effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND VI. p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DOSE - As this compound was insoluble in most common solvents, it was fed in the form of a gum acacia and water suspension. 10 mgs. immediately after the culture injection and 10 mgs. fed 5 hours later.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	X		
	4	X		
Compound Mice	1	X		
	2	X		
	3	X		
	4	X		

X = Death

Compound V1 has no effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate.

DOSE - 10 mgs. in 1 ml. fed immediately after the culture injection, after 5 hours and 24 hours.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	VS[X]		
	3	VS[X]		
	4	S	X	
Compound Mice	1	VS[X]		
	2	VS[X]		
	3	S	X	
	4	S	X	

S = Sick
VS = Very Sick
X = Death

Compound VII has no chemotherapeutic effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND VIII — 4:4'-dinitrodiphenylsulphide

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. immediately after the culture injection, 20 mgs. 5 hours later, and 20 mgs. 24 hours later.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000 mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	VS[X]			
	2	VS[X]			
	3	X			
	4	X			
Compound Mice	1	VS[X]			
	2	VS	X		
	3	X			
	4	X			

VS = Very Sick
X = Death

4:4'-dinitrodiphenylsulphide has no effect on a Bacillus Aertrycke infection of mice.

D. BACILLUS AERTRYCKE

COMPOUND 1X 4:4'-diaminodiphenylsulphide

DOSE - Dilute solution in lactic acid 10 mgs. in 1 ml. fed immediately after the culture injection and 5 hours later.

STRAIN - Bacillus Aertrycke U 18 hour culture emulsified and diluted in 5,000 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection		
		1	2	3
Control Mice	1	X		
	2	X		
	3	VS[X]		
	4	VS	X	
Compound Mice	1	X		
	2	VSx		
	3	VS[X]		
	4	VS	X	

VS = Very Sick
X = Death

4:4'-diaminodiphenylsulphide has no chemotherapeutic effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND X 4:4'-diacetyldiaminodiphenylsulphide

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. in 1 ml. fed immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 1,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	X			
	4	X			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

X = Death

Diacetyldiaminodiphenylsulphide has no effect on mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND XI 3:5-dinitro-4-hydroxybenzenesulphonamide.

DOSE - Fed in a weak solution of sodium bicarbonate. 10 mgs. immediately after the culture injection and again after 5 hours.

STRAIN - Bacillus Aertrycke U
18 hour culture emulsified and diluted 1 in 5,000 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	VS[X]			
	4	VS[X]			
Compound Mice	1	X			
	2	X			
	3	X			
	4	X			

VS = Very Sick
X = Death

3:5-dinitro-4-hydroxybenzenesulphonamide has no effect on a Bacillus Aertrycke infection of mice.

D. BACILLUS AERTRYCKE

COMPOUND XIII 3-nitro-4-aminobenzenesulphonamide

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. per ml. immediately after the culture injection after 5 hours and 24 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 100 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	VF	VF	VF	VF
	4	VF	VF	VF	VF
Compound Mice	1	X			
	2	X			
	3	S	X		
	4	S	VF	VF	VF

VF = Very Fit
S = Sick
X = Death

3-nitro-4-aminobenzenesulphonamide has no effect on a fatal dose of Bacillus Aertrycke in mice

D. BACILLUS AERTRYCKE

COMPOUND XIV — M. & B. 693
2-sulphanilylaminopyridine

DOSE - Feebly soluble in water.
Fed in a solution 20 mgs. per ml. immediately after the culture injection and after 5 and 24 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 100 with mucin 1 ml. injected intraperitoneally.

		Days after Injection				
		1	2	3	4	5
Control Mice	1	X				
	2	X				
	3	VF	VF	VF	VF	VF
	4	VF	VF	VF	VF	VF
Compound Mice	1	X				
	2	X				
	3	S	VF	VF	VF	VF
	4	S	VF	VF	VF	VF

V = Very Fit
S = Sick
X = Death

2-sulphanilylaminopyridine has no chemotherapeutic effect on a lethal dose of Bacillus Aertrycke in mice.

D. BACILLUS AERTRYCKE

COMPOUND XV 1:1-di(p-acetylaminobenzenesulphonyl) piperazine

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. per ml. immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 100 with mucin. 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	VF	VF	VF	VF
	4	VF	VF	VF	VF
Compound Mice	1	X			
	2	X			
	3	S	X		
	4	S	VF	VF	VF

VF = Very Fit
S = Sick
X = Death

Compound XV has no curative effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND XVII Piperidine-1-piperidino-acridone-3-sulphonate.

DOSE - Soluble in water. 10 mgs. per ml. fed immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V 18 hour culture emulsified and diluted 1 in 100 with mucin 1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	VF	VF	VF	VF
	4	VF	VF	VF	VF
Compound Mice	1	X			
	2	X			
	3	VS	X		
	4	VS	X		

VF = Very Fit
VS = Very Sick
X = Death

Compound XVII has no curative effect on a mouse Bacillus Aertrycke infection.

D. BACILLUS AERTRYCKE

COMPOUND XIX 1:4'di-(p-aminobenzenesulphonyl) piperazine.

DOSE - Insoluble in water and thus fed in a gum acacia and water suspension. 20 mgs. per ml. immediately after the culture injection and after 5 hours.

STRAIN - Bacillus Aertrycke V
18 hour culture emulsified and diluted 1 in 100 with mucin.
1 ml. injected intraperitoneally.

		Days after Injection			
		1	2	3	4
Control Mice	1	X			
	2	X			
	3	VF	VF	VF	VF
	4	VF	VF	VF	VF
Compound Mice	1	X			
	2	X			
	3	S	S	X	
	4	S	VF	VF	VF

VF = Very Fit
S = Sick
X = Death

Compound XIX has no curative effect on a Bacillus Aertrycke infection in mice.

PART II

INVESTIGATION OF THE MODE OF ACTION

BACTERIOSTATIC AND BACTERICIDAL ACTION OF VARIOUS COMPOUND ON THE STREPTOCOCCUS, PNEUMOCOCCUS, STAPHYLOCOCCUS AND ON BACILLUS AERTRYCKE

PROCEDURE

Dilutions of the compound under examination were made in papaine broth (to which sterile serum had been added if the compound was to be tested against the haemolytic streptococcus or against the pneumococcus). A weighed amount of the compound was dissolved or suspended in a sterile water and from this solution further dilutions were made.

1 ml. of the solution of the compound was added to 5 mls. of the broth medium and the final concentration of the compound, quoted in the protocols, was that in the broth.

The broth tubes were then inoculated with 0.1 ml. of a thick bacterial suspension (obtained by emulsifying an 18 hour slope culture of the organism) and incubated at 37°C for 18 hours. A control broth tube, with broth and organisms only was included in each series. In order to examine both the bacteriostatic and the bactericidal activity of the compound, sub-cultures from the/

the broth tubes were made on blood agar plates at 3 hour, 6 hour and 18 hour intervals. The blood plates were read for growth after a further 18 hours incubation, and from the extent of growth on these plates the activity of the drug was ascertained.

The same group of compounds as used in the in vivo experiments were tested for their antibacterial activity in the test tube.

The four organisms used in these experiments were the B-haemolytic streptococcus, the pneumococcus, the staphylococcus and Bacillus Aertrycke, and the results obtained are briefly summarised in Table II. The details of the experiments from which these results are abstracted are to be found on pages 150 - 235 and their significance is discussed in Section III (page 251)

IN VITRO EXPERIMENTS

Bacteriostatic & Bactericidal Activity

Compound Number	H. Streptococcus	Pneumococcus	Staphylococcus	Bacillus Aertrycke
1	++	++	0	0
11	+	++	0	0
111	+++	+	0	0
1V	±	+	0	0
V	+++	±	+ to ±	0
V1	+++	+++ to ++	± to 0	+
V11	++	++	± to 0	+
V111	± to 0	±	+	0
1X	++ to +	+	+	0
X	++ to +	0	+	+
X1	0	0	± to 0	0
X11	0	0	0	0
X111	0	0	0	0
X1V	0	0	0	0
XV	0	0	0	0
XV1	+++	± to 0	0	0
XV11	++	+++ to ++	0	0
XV111	-	-	0	0
X1X	0	0	0	0
XX	0	0	0	0
XX1	0	0	0	0
XX11	++	++	0	0

KEY:

+++ = Bactericidal at 1 in 1,000 Bacteriostatic at 1 in 10,000
++ = " " 1 in 100 " " 1 in 1,000
+ = " " 1 in 10 " " 1 in 100
± = Growth retarding effect
0 = No action
- = Not tested

IN VITRO EXPERIMENTS

A. HAEMOLYTIC STREPTOCOCCUS

Compound I p-aminobenzenesulphonamide

DILUTIONS - Diluted 1 in 100 and 1 in 10,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of an 18 hour culture to each tube.

Incubation time before sub-culture	Growth on Blood Agar plates after 18 hours inculbation		
	Dilutions of Compounds		
6	1/100	1/10,000	Control (no compound)
3 hours	0	X	XX
6 hours	0	X	XX
18 hours	0	x	XX

XX = Thick growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound I, p-aminobenzenesulphonamide has a definite bacterostatic and bactericidal effect on the growth of the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND II p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100 and 1 in 10,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture to each tube.

Incubation time before sub-culture	Growth on Blood Agar after 18 hours at 37°C.		
	Dilutions of Compounds		
	1/100	1/10,000	Control (no compound)
3 hours	XX	XX	XX
6 hours	XX	XX	XX
18 hours	0	X	XX

XX = Thick Growth
X = Growth
0 = Sterile

Compound II, p-hydroxybenzenesulphonamide has a bactericidal effect on the growth of the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100 and 1 in 10,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A 0.1 ml. of 18 hour culture in each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/10,000	Control (no compound)
3 hours	x	X	X
6 hours	x	X	X
18 hours	0	0	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

3-nitro-4-hydroxybenzene has a fairly strong growth retarding and killing power on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Comcpounds			
	1/100	1/1,000	1/10,000	Control
3 hours	X	X	X	XX
6 hours	$\underline{x}$	X	XX	XX
18 hours	X	X	X	XX

$\underline{x}$ = 1 - 2 colonies
X = Growth
XX = Thick Growth

3-amino-4-hydroxybenzenesulphonamide has a slight bacteriostatic effect on the growth of the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND $\overline{V}$ p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100, 1 in 10,000 and 1 in 1,000,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	$\frac{1}{100}$	$\frac{1}{10,000}$	$\frac{1}{1,000,000}$	Control
3 hours	$\underline{x}$	$\underline{x}$	XX	XX
6 hours	0	$\underline{x}$	XX	XX
18 hours	0	0	X	XX

XX = Thick Growth
X = Growth
$\underline{x}$ = 1 - 2 colonies
$\overline{0}$ = Sterile

Compound V has a definite bacteriostatic and bactericidal effect on the growth of the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND V1 — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DILUTION - Diluted 1 in 100, 1 in 10,000 and 1 in 1,000,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compounds			
	1/100	1/10,000	1/1,000,000	Control
3 hours	0	X	XX	XX
6 hours	0	x	XX	XX
18 hours	0	0	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound V1 has a definite bacteriostatic and bactericidal action on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate.

DILUTION - Diluted 1 in 100, 1 in 10,000 and 1 in 1,000,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth in Blood Agar — Dilutions of Compound			
	1/100	1/10,000	1/1,000,000	Control
3 hours	x	XX	XX	XX
6 hours	x	XX	XX	XX
18 hours	0	x	X	XX

X = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound VII has a slight bacteriostatic and bactericidal effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND VIII 4:4'-dinitrodiphenylsulphide.

DILUTION - Diluted 1 in 100, 1 in 1,000 and 1 in 10,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A 0.1 ml. 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	0	x	XX	XX
6 hours	x	x	X	XX
18 hours	X	X	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

4:4'-dinitrophenylsulphide has little or no bacteriostatic effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND 1X Diaminodiphenylsulphide

DILUTION - Diluted 1 in 100, 1 in 1000 and 1 in 10,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth in Blood Agar — Dilution of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	x	X	XX
6 hours	x	X	X	XX
18 hours	0	X	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile.

Diaminodiphenylsulphide has a bacteriostatic and a slight bactericidal action on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND X Diacetyldiaminodiphenylsulphide

DILUTION - Diluted 1 in 100, 1 in 1,000 and 1 in 10,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	X	X	XX
6 hours	x	X	X	XX
18 hours	0	X	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Diacetyldiaminodiphenylsulphide has a slight bacteriostatic and bactericidal action on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XI — 3:5-dinitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth tubes.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	x	X	XX
6 hours	x	x	X	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

3:5-dinitro-4-hydroxybenzenesulphonamide has little bacteriostatic effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XII — Methylquinolylaminobenzenesulphonamide.

DILUTION - Diluted 1 in 100.

STRAIN - Haemolytic Streptococcus E.

0.1 ml. 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound	
	$\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XII has no *in vitro* effect on the growth of the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XIII — 3-nitro-4-aminobenzenesulphonamide.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptocccus G. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound	
	$\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

3-nitro-4-aminobenzenesulphonamide has no bactericidal effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XlV — 2-sulphanilylaminopyridine (M. & B. 693)

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptococcus G. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

2-sulphanilylaminopyridine has no effect on the growth of the haemolytic streptococcus in vitro.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XV 1:4-di(p-acetylaminobenzenesulphonyl) piperazine.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptococcus G. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution	Control
3 hours	X	XX
18 hours	XX	XX

XX = Thick Growth
X = Growth

Compound XV has no bactericidal effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XVI p-diazobenzenesulphonamide.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compounds			
	1/100	1/1,000	1/10,000	Control
3 hours	x	x	x	XX
6 hours	0	0	0	XX
18 hours	0	0	0	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile.

p-diazobenzenesulphonamide has a bactericidal effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XVII — Piperidine-1-piperidino-acridone-3-sulphonate.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10^0,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on red Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	0	x	X	XX
6 hours	0	x	X	XX
18 hours	0	0	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound XVII has a bacteriostatic and bactericidal effect on the haemolytic streptococcus.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XIX , 1:4-di(p-aminobenzenesulphonyl) piperazine.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptococcus H. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution	Control
3 hours	XX	XX
18 hours	X	XX

XX = Thick Growth
X = Growth

Compound XIX has no effect on the growth of the haemolytic streptococcus *in vitro*.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptococcus H. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX has no effect on the growth of the haemolytic streptococcus *in vitro*.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XX1 Ethyl-4-aminobenzenesulphonyl piperazine-1-carboxylate.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Haemolytic Streptococcus H. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX1 has little effect on the growth of the haemolytic streptococcus in the test tube.

A. HAEMOLYTIC STREPTOCOCCUS

COMPOUND XXII — Sulphonamide coupled with hydroxy arsonic acid.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Haemolytic Streptococcus A. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	0	0	x	XX

XX = Thick Growth
x = 1 - 2 colonies
0 = Sterile

Compound XXII has a slight bactericidal effect on the haemolytic streptococcus.

PART II 1. BACTERIOSTATIC AND BACTERICIDAL EXPERIMENTS.

B. PNEUMOCOCCUS

COMPOUND 1 p-aminobenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus Z.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	X	X	XX
6 hours	0	x	X	XX
18 hours	0	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

p-aminobenzenesulphonamide has a definite bactericidal and bacteriostatic effect on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND II p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus R. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	X	X	X	X
18 hours	0	0	XX	XX

XX = Thick Growth
X = Growth
0 = Sterile

p-hydroxybenzenesulphonamide has a bacteriostatic and a bactericidal action against the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus Z. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	X	X	XX
6 hours	x	X	X	XX
18 hours	0	X	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

3-nitro-4-hydroxybenzenesulphonamide has a bacteriostatic and a bactericidal action on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus Z. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	X	X	XX	XX
6 hours	x	x	XX	XX
18 hours	0	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

3-amino-4-hydroxybenzenesulphonamide has a bacteriostatic and a bactericidal effect on the p neumococcus.

B. PNEUMOCOCCUS

COMPOUND V — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus Y
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilution of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	X	X	XX	XX
18 hours	X	X	XX	XX

XX = Thick Growth
X = Growth

Compound V has slight bacteriostatic action on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND VI — p-aminobenzenesulphonamide coupled with 8-hydroxy quinoline.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus X
0.1 ml. of 18 hour culture added to each tube.

Incubation time before subculture	Growth on Blood Agar — Dilution of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	0	x	X	XX
18 hours	0	0	x	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound VI has a fairly strong bacteriostatic and bactericidal effect on the pneumococcus.

B. Pneumococcus

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus X 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compounds			
	1/100	1/1,000	1/10,000	Compound
3 hours	0	x	X	XX
18 hours	0	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound VII has a definite bacteriostatic and bactericidal action on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND VIII 4:4'dinitrodiphenylsulphide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus Q
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compounds			
	1/100	1/1,000	1/10,000	Compound
3 hours	0	X	XX	XX
18 hours	x	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

4:4'dinitrodiphenylsulphide has a slight bacteriostatic effect on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND IX 4:4'diaminodiphenylsulphide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus P
0.1 ml. of 18 hour culture added toeach tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Compound
3 hours	X	X	XX	XX
18 hours	0	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

4:4'diaminodiphenylsulphide has some bactericidal and bacteriostatic action on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND X 4:4'diacetyldiaminodiphenylsulphide

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilutions of Compounds	
	$\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

4:4'diacetyldiaminodiphenylsulphide has no effect on the growth of the pneumococcus in the test tube.

B. PNEUMOCOCCUS

COMPOUND XI — 3:5-dinitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile serum broth.

STRAIN - Pneumococcus P.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compounds		
	1/100	1/1,000	Control
3 hours	XX	XX	XX
18 hours	X	X	XX

XX = Thick Growth
X = Growth

3:5-dinitro-4-hydroxybenzenesulphonamide has little effect on the growth of the pneumococcus *in vitro*.

B. PNEUMOCOCCUS

COMPOUND XII. Methylquinolylbenzenesulphonamide

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Methylquinolylbenzenesulphonamide has no effect on the growth of the pneumococcus in the test tube.

B. PNEUMOCOCCUS

COMPOUND XIII — 3-nitro-4-aminobenzenesulphonamide.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XIII has no effect on the growth of the pneumococcus in the test tube.

B. PNEUMOCOCCUS

COMPOUND XIV — 2-sulphanilylaminopyridine

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XIV has no effect on the *in vitro* growth of the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND XV — 1:4'di(p-acetylaminobenzenesulphonyl) piperazine.

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth in Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XV has no effect on the *in vitro* growth of the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND XVI — p-aminobenzenesulphonamidediazo-compound.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile serum broth.

STRAINS - Pneumococcus P

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compounds		
	1/100	1/1,000	Control
3 hours	x	X	XX
18 hours	X	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies,

Compound XVI has a feeble bacteriostatic effect on thegrowth of the pneumococcus in the test tube.

B. PNEUMOCOCCUS

COMPOUND XVII — Piperidine-1-piperidino-acridone-3-sulphonate.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile serum broth.

STRAIN - Pneumococcus P. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	X	X	XX
18 hours	0	0	XX

XX = Thick Growth
X = Growth
0 = Sterile

Compound XVII has a bactericidal effect on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND XIX — 1:4-di-(p-aminobenzenesulphonyl) piperazine

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XIX has no bacteriostatic or bactericidal effect on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl piperazine-1-carboxylate

DILUTION — Diluted 1 in 100 in sterile serum broth.

STRAIN - Pneumococcus R

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX has no growth retarding effect on the pneumococcus.

B. PNEUMOCOCCUS

COMPOUND XXII — p-aminobenzenesulphonamide coupled with hydroxy arsonic acid.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Pneumococcus X

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	0	0	0	XX
18 hours	0	X	XX	XX

XX = Thick Growth
X = Growth
0 = Sterile

Compound XXII has a definite bactericidal and bacteriostatic action on the pneumococcus.

PART II BACTERIOSTATIC AND BACTERICIDAL EXPERIMENTS

C. STAPHYLOCOCCUS

COMPOUND I p-aminobenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile serum broth.

STRAIN - Staphylococcus N and M 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
6 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

p-aminobenzenesulphonamide has no effect on a staphylococcal growth in vitro.

C. STAPHYLOCOCCUS

COMPOUND II p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Staphylococcus N
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

p-hydroxybenzenesulphonamide has no effect, bacteriostatic or bactericidal, on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in broth.

STRAIN - Staphylococcus M and N

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth in Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

3-nitro-4-hydroxybensenesulphonamide has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Staphylococcus M and N 0.1 ml. of 18 hour culture added to each tube.

Incubattion time before sub-culture	Growth on Blood Agar — Dilutions of Compounds			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

3-amino-4-hydroxybenzenesulphonamide has no effect on the growth of the staphylococcus in the test tube.

C.? STAPHYLOCOCCUS

COMPOUND V — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100 and 1 in 10,000 in sterile broth.

STRAIN - Staphylococcus N

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/10,000	Control
3 hours	x	XX	XX
18 hours	0	XX	XX

XX = Thick Growth
x = 1 - 2 colonies
0 = Sterile

Compound V has a slight bacteriostatic and bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND VI — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Staphylococcus N
0.1 ml, of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	X	X	XX	XX
18 hours	x	x	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies

Compound VI has a slight bacteriostatic effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate

DILUTION - Diluted 1 in 100 and 1 in 10^{0},000 in sterile broth.

STRAIN - Staphylococcus N

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	$\frac{1}{100}$	$\frac{1}{10,000}$	Control
3 hours	x	X	XX
18 hours	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies

Compound VII has a slight bacteriostatic and bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND VIII 4:4'dinitrodiphenylsulphide

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Staphylococcus N
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	$\frac{1}{100}$	$\frac{1}{1,000}$	Control
3 hours	x	X	XX
18 hours	0	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

4:4'dinitrodiphenylsulphide has a bacteriostatic anda bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND IX 4:4'diaminodiphenylsulphide

DILUTION - Diluted 1 in 100 and 1 in 10^0,000 in sterile broth

STRAIN - Staphylococcus N
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	$\frac{1}{100}$	$\frac{1}{10^0,000}$	Control
3 hours	X	XX	XX
18 hours	0	XX	XX

XX = Thick Growth
X = Growth
0 = Sterile

4:4'diaminodiphenylsulphide has a slight action on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND X 4:4'diacetyldiaminodiphenylsulphide.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus M
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	0	XX

XX = Thick Growth
0 = Sterile

4:4'diacetyldiaminodiphenylsulphide has a bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND XI — 3:5 dinitro-4-hydroxybenzene-sulphonamide

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Staphylococcus N. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	XX	XX	XX
18 hours	x	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2mcolonies

3:5dinitro-4-hydroxybenzenesulphonamide has little bacteriostatic or bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND XII Methylquinolylbenzenesulphonamide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus N.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Methylquinolylbenzenesulphonamide has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XIII — 3-nitro-4-aminobenzenesulphonamide.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

, 3-nitro-4-hydroxybenzenesulphonamide has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XIV — 2 sulphanilylaminopiperazine (M. & B. 693)

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	X X	X X
18 hours	X X	X X

XX - Thick Growth

2-sulphanilylaminopiperazine has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XV — 1:4'di-(p-acetylaminobenzene-sulphonyl)piperazine

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XV has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XVI — p-aminobenzenesulphonamide diazo compound.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Staphylococcus M

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	x	X	XX
18 hours	XX	XX	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies

p-aminobenzenesulphonamide diazo compound has little effect on the growth ofthe staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XVII Piperidine-1-piperidino-acridone-3-sulphonate.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Staphylococcus M.

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	XX	XX	XX
18 hours	XX	XX	XX

XX - Thick Growth

Compound XVII has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XVIII — 4-4'dimethyldiquinolyl 2-2' sulphide.

DILUTION - Diluted 1 in 100, 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Staphylococcus M. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

Compound XVIII has no bacteriostatic or bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND XIX 1:4'di-(p-aminobenzenesulphonyl) piperazine.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XlX has no bacteriostatic or bactericidal effect on the staphylococcus.

C. STAPHYLOCOCCUS

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K. 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XXI — Ethyl-4-aminobenzenesulphonyl piperazine-1-carboxylate.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Staphylococcus K.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX1 has no effect on the growth of the staphylococcus in the test tube.

C. STAPHYLOCOCCUS

COMPOUND XXII — p-aminobenzenesulphonamide coupled with Arsonic Acid.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Staphylococcus N.
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	X	XX	XX	XX

XX = Thick Growth
X = Growth.

Compound XXII has no effect on the growth of the staphylococcus in the test tube.

PART II BACTERIOSTATIC AND BACTERICIDAL EXPERIMENTS

D. BACILLUS AERTRYCKE

COMPOUND 1 p-aminobenzenesulphonamide

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke U and V 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compounds		
	1/100	1/1,000	Control
3 hours	XX	XX	XX
18 hours	XX	XX	XX

XX = Thick Growth

p-aminobenzenesulphonamide has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND II p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke V
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	$\frac{1}{100}$	$\frac{1}{1,000}$	Control
4 hours	XX	XX	XX
18 hours	XX	XX	XX

XX = Thick Growth

p-hydroxybenzenssulphonamide has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND III — 3-nitro-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke U

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	XX	XX	XX
18 hours	XX	XX	XX

XX = Thick Growth

3-nitro-4-hydroxybenzenesulphonamide has no bacteriostatic or bactericidal effect on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND IV 3-amino-4-hydroxybenzenesulphonamide.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
6 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

3-amino-4-hydroxybenzenesulphonamide has little effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND V — p-aminobenzenesulphonamide coupled with p-hydroxybenzenesulphonamide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound V has no bacteriostatic or bactericidal effect on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND VI — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke U 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	x	X	XX
18 hours	0	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound VI has a slight bacteriostatic and bactericidal effect on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND VII — p-aminobenzenesulphonamide coupled with 8-hydroxyquinoline methosulphate.

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	x	X	XX
18 hours	0	X	XX

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

Compound VII has a slight bacteriostatic and bactericidal effect on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND VIII 4:4'dinitrodiphenylsulphide

DILUTION - Diluted 1 in 100 and 1 in 1,000 in sterile broth.

STRAIN - Bacillus Aertrycke U 0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound		
	1/100	1/1,000	Control
3 hours	X X	XX	XX
18 hours	XX	XX	XX

XX = Thick Growth

4:4'dinitrodiphenylsulphide has no action on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND IX 4:4'diaminodiphenylsulphide

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar		
	$\frac{1}{100}$	$\frac{1}{1,000}$	Control
3 hours	XX	XX	XX
18 hours	XX	XX	XX

XX = Thick Growth

4:4'diaminodiphenylsulphide has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND X — 4:4'diacetyldiaminodiphenylsulphide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	0	XX

XX = Thick Growth
0 = Sterile

4:4'diacetyldiaminodiphenylsulphide has a bactericidal effect on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND XI — 3:5 dinitro-4-hydroxybenzene-sulphonamide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

3:5 dinitro-4-hydroxybenzenesulphonamide has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XII — Methylquinolylaminobenzenesulphonamide.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX - Thick Growth

Methylquinolylbenzenesulphonamide has no effect on the growth of Bacillus Aertrycke *in vitro*.

D. BACILLUS AERTRYCKE

COMPOUND XIII 3-nitro-4-aminobenzenesulphonamide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V
0.1 ml. of 18 hour culture added to each tube.

Incubation time before subculture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XIII has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XIV 2-sulphanilylaminopyridine

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke W

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

2-sulphanilylaminopyridine has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XV — 1-4'di-(p-acetylaminobenzene-sulphonyl)piperazine

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XV has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XVI p-aminobenzenesulphonamide diazo compound.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

q

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

Compound XVI has little action on the growth of Bacillus Aertrycke *in vitro*.

D. BACILLUS AERTRYCKE

COMPOUND XVII — Piperidine-1-piperidino-acridone-3-sulphonate.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar			
	Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	XX	XX	XX	XX
18 hours	XX	XX	XX	XX

XX = Thick Growth

Compound XVII has no action on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XVIII 4:4'dimethyldiquinolyl-2-2'sulphide

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

4:4'dimethyldiquinolyl 2-2'sulphide has no effect on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XlX — 1:4 di-(p-aminobenzenesulphonyl) piperazine.

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XlX has no bacteriostatic or bactericidal action on Bacillus Aertrycke.

D. BACILLUS AERTRYCKE

COMPOUND XX — Ethyl-4-acetylaminobenzenesulphonyl-piperazine-1-carboxylate

DILUTION - Diluted 1 in 100 in sterile broth.

STRAIN - Bacillus Aertrycke V

0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XX has no action on the growth of Bacillus Aertrycke in the test tube.

D. BACILLUS AERTRYCKE

COMPOUND XXI — Ethyl-4-aminobenzenesulphonyl-piperazine-1-carboxylate

DILUTION - Diluted 1 in 100 in sterile serum broth.

STRAIN - Bacillus Aertrycke V
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar	
	Dilution $\frac{1}{100}$	Control
3 hours	XX	XX
18 hours	XX	XX

XX = Thick Growth

Compound XXI has little effect on the growth of Bacillus Aertrycke *in vitro*.

D. BACILLUS AERTRYCKE

COMPOUND XXII — p-aminobenzenesulphonamide coupled with Arsonic Acid.

DILUTION - Diluted 1 in 100; 1 in 1,000 and 1 in 10,000 in sterile broth.

STRAIN - Bacillus Aertrycke U
0.1 ml. of 18 hour culture added to each tube.

Incubation time before sub-culture	Growth on Blood Agar — Dilutions of Compound			
	1/100	1/1,000	1/10,000	Control
3 hours	x	XX	XX	XX
18 hours	x	XX	XX	XX

XX = Thick Growth
x = 1 - 2 colonies.

Compound XXII has a slight growth retarding effect on the Bacillus Aertrycke.

PART II

INVESTIGATION OF MODE OF ACTION

2. EFFECT OF THE VARIATION OF THE pH OF THE MEDIUM ON THE ACTION OF SULPHANILAMIDE.

In order to determine the effect of a change in the pH of the medium in which the organisms are growing on the bactericidal action of sulphanilamide, varying amounts of acid and alkali were added to tubes of broth, these tubes were inoculated with streptococci, and a known concentration of sulphanilamide added. After incubatio n for 18 hours the pH of each tube was determined and sub-cultures from the tubes were made on blood agar. From the growth on the blood agar the extent of bactericidal activity at a known pH was determined. A similar experiment was carried out with the Pneumococcus and with the Staphylococcus.

I STREPTOCOCCUS AND PNEUMOCOCCUS Strain B & X 18 hour cultures on agar. Test carried out in serum papain broth emulsified and 0.1ml. added to each tube.

Compound: p-aminobenzenesulphonamide.
Diluted in sterile distilled water 1 in 100 and 1 in 10,000.

(a) Incubated for 2 hours and plated on blood agar.

pH	Sulphanilamide	Streptococcus B	Pneumococcus X
8.3	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	x	x
	0 (Control)	XX	X
7.2	$\frac{1}{100}$	x	0
	$\frac{1}{10,000}$	X	x
	0 (Control)	XX	X
6.8	$\frac{1}{100}$	x	0
	$\frac{1}{10,000}$	X	0
	0 (Control)	XX	X
6.4	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	x	0
	0	XX	0

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

(b) Incubated for 18nhours and plated on blood agar.

pH	Sulphanilamide	Streptococcus B	Pneumococcus X
8.3	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	0	0
	0 (Control)	XX	x
7.2	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	0	0
	0 (Control)	XX	x
6.8	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	0	0
	0	XX	0
6.4	$\frac{1}{100}$	0	0
	$\frac{1}{10,000}$	0	0
	0 (Control)	XX	0

XX= Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

II HAEMOLYTIC STREPTOCOCCUS, PNEUMOCOCCUS AND STAPHYLOCOCCUS Strains A, X and N

18 hour cultures on agar emulsified and 0.1 ml. added to each tube of serum papain broth.

Compound: p-aminobenzenesulphonamide.

Incubated for 18 hours and plated on blood agar.

pH range 8.3 - 4.1

pH	Streptococcus Strain A		Pneumococcus Strain X		Staphylococcus Strain N	
	Alone	1/100 Sulphanilamide	Alone	1/100 Sulphanilamide	Alone	1/100 Sulphanilamide.
8.3	X	0	XX	0	XX	XX
7.5	XX	x	XX	0	XX	XX
7.1	X	x	XX	0	XX	XX
6.8	0	x	X	0	XX	XX
6.1	x	0	X	0	XX	XX
5.6	x	0	0	0	XX	XX
4.8	0	0	0	0	X	X
4.1	0	0	0	0	0	0

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

III HAEMOLYTIC STREPTOCOCCUS, PNEUMOCOCCUS AND STAPHYLOCOCCUS Strains A, Q and N

18 hour cultures emulsified and 0.1 ml. added to each tube of serum papain broth.

Compound: p-aminobenzenesulphonamide

Incubated for 18 hours and plated on blood agar.

pH = 8.4 - 4.0

pH	Streptococcus Strain A		Pneumococcus Strain Q		Staphylococcus Strain N	
	Alone	1/100 Sulphanilamide	Alone	1/100 Sulphanilamide	Alone	1/100 Sulphanilamide.
8.4	XX	0	XX	0	XX	XX
7.6	XX	0	XX	0	XX	XX
7.2	XX	0	XX	0	XX	XX
6.8	XX	0	X	0	XX	XX
6.0	XX	0	X	0	XX	XX
5.3	0	0	0	0	XX	XX
5.0	0	0	0	0	XX	XX
4.6	0	0	0	0	X	x
4.0	0	0	0	0	X	0

XX = Thick Growth
X = Growth
x = 1 - 2 colonies
0 = Sterile

From these results it appears that in each case any variation in the bactericidal and the bacteriostatic action of the sulphanilamide seems to be due to the direct effect of the change in the pH on the viability of the organisms (as shown by the control experiments) and not to the drug itself.

PART II INVESTIGATION OF THE MODE OF ACTION

3. EFFECTS OF VARYING THE OXIDATION REDUCTION POTENTIAL

In view of the fact that the bactericidal action of these compounds is very low compared with their curative effect in vivo, the possibility arises that the action in vivo takes place under conditions of oxidation-reduction potential rather different from those which obtain in the living organism.

Thus a series of experiments was carried out to determine the bacteriostatic and bactericidal action of p-aminobenzenesulphonamide using media poised at different oxidation-reduction levels.

Alterations in the oxidation reduction level were made by means of ascorbic acid and by thioglycollic acid. A solution of ascorbic acid or thioglycollic acid neutralised by means of sodium carbonate was added in different amounts to serum containing different dilutions of sulphanilamide broth. A suspension of actively growing streptococci was then added and the tubes were incubated for 24 hours. At the end of that time sub-cultures were made on blood agar plates and the Eh of the broth cultures was determined by means of a series of oxidation-reduction indicators:

phenol-indo-2:6 dichlorophenol

O-cresol-indo-2:6 dichlorophenol

brilliant cresyl blue

methylene blue

potassium indigo tetrasulphonate

potassium indigo disulphonate

phenosafranine

neutral red

The pH remained at about 7.6 throughout the experiments.

A. ASCORBIC ACID Eh range -0.046 to 0.217

SERIES 1	1	2	3	4	5	6
Ascorbic acid in mgs.	75	50	25	10	5	0
Sulphanilamide	0	0	0	0	0	0
Streptococcal suspension in mls.	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 4 hours	XX	XX	XX	XX	XX	XX
Plated after 24 hours	XX	XX	XX	XX	XX	XX
pH readings after 24 hours	7.6	7.6	7.6	7.6	7.6	7.6
Eh after 24 hours	-0.046	0.011	0.047	0.047	0.115	0.217

XX = Thick Growth

ASCORBIC ACID Eh range -0.046 to 0.217

SERIES 11	1	2	3	4	5	6
Ascorbic acid in mgs.	75	50	25	10	5	0
Sulphanilamide concentration in media	1/100	1/100	1/100	1/100	1/100	1/100
Streptococci in mls.	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 4 hours	0	0	0	0	0	0
Plated after 24 hours	x	x	0	x	x	0
pH readings after 24 hrs.	7.6	7.6	7.6	7.6	7.6	7.6
Eh after 24 hours	-0.046	0.011	0.047	0.047	0.115	0.217

0 = Sterile culture

x = 1 or 2 colonies.

ASCORBIC ACID Eh range-0.046 to 0.217

SERIES 111	1	2	3	4	5	6
Ascorbic acid in mgs.	75	50	25	10	5	0
Sulphanilamide concentration in media	1/1000	1/1000	1/1000	1/1000	1/1000	1/1000
Streptococci in mls.	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 4 hours	XX	XX	XX	XX	XX	0
Plated after 24 hours	XX	XX	XX	XX	XX	XX
pH readings after 24 hrs.	7.6	7.6	7.6	7.6	7.6	7.6
Eh after 24 hours	-0.046	0.011	0.047	0.047	0.115	0.217

XX = Thick Growth
X = 1 or 2 colonies
0 = Sterile culture

B. THIOGLYCOLLIC ACID Eh range -0.252 to 0.217

SERIES	1	2	3	4	5	6	7
Thioglycollic acid in mls.	1.0	0.8	0.6	0.4	0.2	0.1	0.0
Sulphanilamide	0	0	0	0	0	0	0
Streptococcal suspension in mls.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 3 hours	XX	XX	XX	XX	XX	XX	XX
P Plated after 24 hours	XX	XX	XX	XX	XX	XX	XX
pH after 24 hours	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Eh after 24 hours	-0.252	-0.252	-0.125	-0046	0.011	0.119	0.219

XX = Thick Growth

B. THIOGLYCOLLIC ACID Eh range -0.252 to 0.217

SERIES 11	1	2	3	4	5	6	7
Thioglycollic acid in mls.	1.0	0.8	0.6	0.4	0.2	0.1	0.0
Concentration of Sulphanilamide in mls.	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$
Streptococcal susoension in mls.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 3 hours	X	X	X	X	X	X	X
Plated after 24 hours	0	0	0	0	0	$\underline{x}$	0
pH after 24 hours	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Eh after 24 hours	-0.252	-0.252	-0.125	-0.046	0.011	0.119	0.219

X = Growth
$\underline{x}$ = 1 or 2 colonies
0 = Sterile culture

B. THIOGLYCOLLIC ACID Eh range -0.252 to 0.217

SERIES 111	1	2	3	4	5	6	7
Thioglycollic acid in mls.	1.0	0.8	0.6	0.4	0.2	0.1	0.0
Concentration of Sulphanilamide in mls.	$\frac{1}{1000}$	$\frac{1}{1000}$	$\frac{1}{1000}$	$\frac{1}{1000}$	$\frac{1}{1000}$	$\frac{1}{1000}$	$\frac{1}{1000}$
Streptococcal suspension in mls.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Plated after 3 hours	XX	XX	XX	XX	XX	XX	XX
Plated after 24 hours	XX	XX	XX	XX	XX	XX	XX
pH after 24 hours	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Eh after 24 hours	-0.252	-0.252	-0.125	-0.046	0.011	0.119	0.219

XX = Thick Growth

The conclusion derived from these experiments is that alterations in the oxidation-reduction level within the limits of these experiments, appear to have no influence on the bactericidal and bacteriostatic action of sulphanilamide in vitro.

PART II

INVESTIGATION OFTHE MODE OF ACTION

4. POSSIBILITY OF THE FORMATION OF OXIDATION PRODUCTS FROM SULPHANILAMIDE.

One theory which has been put forward to explain the activity of sulphanilamide is that in the animal body it is changed to some substance of a much greater bactericidal power. The French investigator, Mayer (1937) holds that the active agent is p-hydroxyaminobenzenesulphonamide while Domagk and co-workers think that p-nitrobenzene-sulphonamide is the active agent. Both these views agree in suggesting that the hypothetical active compound is formed by oxidation of the original substance. Attempts were therefore made to ascertain whether a bactericidal compound could be produced in vitro by oxidation of p-amino benzenesulphonamide.

INCUBATION OF SULPHANILAMIDE WITH LIVER MUSH

The following experiment was carried out in the attempt to oxidise sulphanilamide by oxygen in presence of the enzymes of liver tissue.

The liver was removed from a mouse with aseptic precautions, ground up with sterile sand and saline, and the liver mush so obtained incubated for/

for 18 hours at 37°C (1) with a known concentration of sulphanilamide (2) with the concentration of sulphanilamide and ascorbic acid and (3) with ascorbic acid alone. The same experiment was carried out both with and without aeration with sterile air.

Dilutions of the three solutions:

(1) Sulphanilamide

(2) Sulphanilamide plus Ascorbic acid

(3) Ascorbic Acid,

all after incubation with liver mush, both with and without aeration, were then tested for their antibacterial power, and compared with simple concentrations of the drug and Ascorbic Acid alone. This experiment was carried out with the streptococcus, with the pneumococcus, with the staphylococcus and with Bacillus Aertrycke, and as in no case was there any increase in the antibacterial power, no active compound was developed during the experiment.

INCUBATION OF SULPHANILAMIDE WITH HYDROGEN PEROXIDE

A further experiment to try to isolate some active intermediate compound from sulphanilamde was carried out using hydrogen peroxide as the oxidising agent. A known concentration of sulphanilamide was incubated with hydrogen peroxide and/

and a trace of manganous sulphate (as a catalyst, manganese is known to be a carrier of H_2O_2), with the hydrogen peroxide alone, and with a trace of manganous sulphate alone. The tubes with these solutions were evacuated to dryness in vacuo and sufficient distilled water added to the residue to give the original concentration of sulphanilamide. A similar control experiment was carried out with aniline sulphate in place of the sulphanilamide. The sulphanilamide treated with hydrogen peroxide and manganous sulphate, and the aniline sulphate similarly treated, were then compared with fresh solutions of sulphanilamide and aniline sulphate for their bactericidal power. This experiment was repeated several times and from the results obtained using the streptococcus as the test organism, it was concluded that no very active antistreptococcal compound was formed in these experiments.

In as far as these investigations gave negative results they were against the theory of Mayer and others, though of course the possibility exists that under conditions not yet investigated the hypothetical intermediate compound might be produced.

SECTION III DISCUSSION.

Before discussing the above experimental results in detail it is perhaps desirable to refer to certain limitations of the work and some special difficulties which were encountered.

In the animal experiments with all four organisms certain difficulties were encountered in connection with the standardisation of the lethal dose of the bacterium. These experiments were carried out over a period of two years, and frequent sub-culture of the organisms and storage on artificial media caused a certain loss of virulence of the various strains. Though the virulence could generally be increased again by animal passage, in some cases it was difficult to raise it sufficiently for practical work, and such strains had to be discarded. Thus, every compound was not tested with the same strain of a particular organism.

Hog's gastric mucin made up in a 5% suspension was in many cases used to increase the virulence of the haemolytic streptococcus, the staphylococcus and of Bacillus Aertrycke. The exact nature of the part played by this material in augmenting the lethal action of the bacteria is a matter/

matter of some doubt, and though the introduction of another extraneous and variable factor is obviously undesirable, with organisms such as the staphylococcus it is unavoidable. Mucin controls were of course included in each experiment. On some occasions additional difficulties were encountered owing to the toxic action of mucin which had been kept for some time, but satisfactory results were obtained by the use of only fresh solutions. The cause of this curious deterioration of the mucin was not discovered. Experiments carried out to test its sterility showed that there was no contamination by bacteria or fungi. However, three separate samples developed this toxicity in each case after the solution had stood for several weeks. Fresh solutions were always inocuous.

The relative insolubility of some compounds in a neutral medium and the consequent employment of suspensions resulted in certain limitations of accuracy of dosage in these cases. In a few instances it was even difficult to prepare a suitable suspension but even in such cases it was found possible to make the dosage sufficiently accurate for the purpose. The consistency of the results in a number of animals is an indication of this in the case of the therapeutically active substances.

With the soluble compounds accurate control of dosage was naturally a much simpler matter.

The relation insolubility of some compounds also gave rise to difficulties in the in vitro experiments.

Unfortunately it was necessary to limit the testing of compounds in vivo on the pneumococcus and it has not yet been possible to test the last ten compounds on this organism.

It may be observed that in all the experiments the compounds were administered orally to the mice. Actually in the case of sulphanilamide certain experiments were carried out using the subcutaneous method but the protection afforded appeared to be less. This is in accordance with various observations in the literature. (Barlow (1937)) It is perhaps rather surprising that insoluble compounds with relatively large molecules like diaminodiphenylsulphide are active when administered by mouth as difficulties of absorption might be expected.

DISCUSSION OF THE ACTIVITY OF VARIOUS COMPOUNDS ON DIFFERENT BACTERIA.

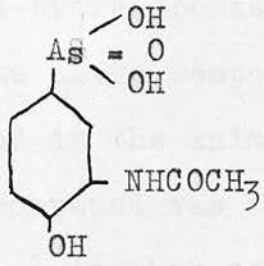
Many attempts have been made to discover a direction in which to modify the structure of the simple p-aminobenzenesulphonamide, the general aim being to try to obtain new compounds with the lowest possible/

possible toxicity influencing the greatest number of bacterial diseases, and especially a compound of low toxicity having a curative action against pneumococcal and staphylococcal infections.

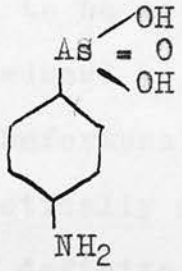
A consideration of the results obtained with various new compounds on streptococcal infections reveals that one of the most interesting results of the investigations described is the relatively high antistreptococcal activity of 3-nitro-4-hydroxybenzenesulphonamide. All the sulphur containing compounds 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, eother free or substituted in the para position in the benzene ring. The activity of this 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-hydroxy grouping. It will also be noted that the toxicity of 3-nitro-4-hydroxybenzenesulphonamide is slightly less than that of sulphanilamide.

If one attempts to draw an analogy between the antistreptococcal drusg of the salvarsan group, where/

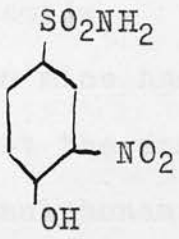
where the element arsenic plays the central role and the antibacterial drugs of the sulphanilamide group where the element sulphur takes the place of arsenic, the relation of 3-nitro-4-hydroxybenzenesulphonamide to p-aminobenzenesulphonamide is somewhat analogous to that of stovarsol to atoxyl (p-amino arsonic acid).



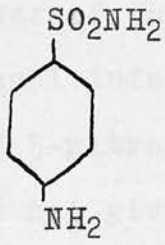
Stovarsol



Atoxyl



3-nitro-4-hydroxybenzene-sulphonamide



Sulphanilamide

In the case of the arsenicals stovarsol has a hydroxyl group in the para position and a nitrogen containing group, an acetyl amino group, in the meta position to the arsenic while atoxyl has an amino group in the para position.

Correspondingly in the case of the sulphanilamide derivatives where sulphur takes the place of arsenic, 3-nitro-4-hydroxybenzenesulphonamide resembles/

resembles stovarsol in having a hydroxyl group in the para position and a nitrogen containing group, the nitro group, in the meta position to the sulphur while sulphanilamide like atoxyl has an amino group in the para position.

For a close analogy one would expect 3-amino-4-hydroxybenzenesulphonamide to be active and that the nitro compound would be reduced to the amino compound in the animal body. Unfortunately, the amino compound was found to be practically inactive.

Another compound showing definite anti-streptococcal activity is 3-nitro-4-aminobenzene-sulphonamide. The curative power of this compound in mice haemolytic streptococcal infections is of almost the same value as that of 3-nitro-4-hydroxy-benzenesulphonamide, though it does not give complete protection, and mice which have apparently recovered may die after several weeks from a haemolytic streptococcal infection. It is interesting to note that Trefouël et al. (1937) reported this compound inactive.

During the course of these investigations it has fairly often been found that mice which have apparently recovered as the reult of treatment with one of these compounds may suffer from recurring or chronic infections. Such mice may die from the original infection after 6 to 8 weeks and a pure culture/

culture can generally be isolated from the organs, or if the mice are killed at the end of this time a chronic infection may be found. Thus a compound found to be apparently therapeutically active may in fact only postpone death for several weeks or convert an acute condition into a low grade chronic one. These recurring infections were found in several experiments with the streptococcus and also with the staphylococcus.

Two other compounds showing definite anti-streptococcal activity are 1:4 di(p-aminobenzene-sulphonyl) piperazine and its acetyl derivative, 1:4(di p-acetylaminobenzenesulphonyl) piperazine. The first compound may be regarded as two molecules of sulphanilamide joined by two ethylene linkages, while in the second, two acetyl groups have been introduced in addition. It is therefore perhaps not surprising that they should be chemotherapeutically active. It is interesting to compare the structure of these two compounds with that of diaminodiphenyl-sulphone and its acetyl derivative, diacetyldiamino-diphenylsulphone. In all four compounds, the grouping SO_2-C_6H_4-NH_2 or SO_2-C_6H_4-NH,Ac is very prominent. The piperazine compounds are however much less toxic than the sulphones, and also it would seem less active.

M. & B. 693 (2-sulphanilylaminopyridine) was found to have a strong curative action against mouse streptococcal septicaemias, 100 per cent of the infected animals recovering completely. The toxicity of this compound for mice is about 100 mgs. per 20 gram mouse, that is slightly less toxic than sulphanilamide.

In the case of pneumococcal infections a slight death delaying action was found using some of the 13 substances tested but no true protection power was shown by any one of them. The antipneumococcal action of M.& B. 693 was not examined.

In the case of staphylococcal infections in mice treated with the series of compounds almost uniformly negative results were obtained with one or two exceptions, Thus, 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. Another compound showing a slight antistaphylococcal action was 1:4 di(p-acetylaminosulphonyl) piperazine which with a

dose/

dose of organisms killing 75 per cent of the mice in the control group, showed a definite protective action for the treated mice. It is rather surprising that this compound and 4:4'-diacetyldiaminodiphenylsulphide, both being acetyl derivatives of inactive compounds, should themselves show a slight degree of activity. In the case of the action of sulphanilamide on the streptococcus the acetyl derivative has in general been shown to be very much less active than the parent base. A third compound showing a slight protective action on staphylococcal infections in mice was M. & B. 693 (2-sulphanilylaminopyridine). As this compound has been used with success, clinically in staphylococcal infections (Fenton & Hodgkiss,1938) it may be expected to show some death delaying action on staphylococcal infections in experimental animals. From the 16 compounds examined for therapeutic activity against Bacillus Aertrycke infections of mice only one, p-aminobenzenesulphonamide had any action, and that was only to delay death of the infected animals by some hours.

ACTION OF COMPOUNDS ON BACTERIA IN VITRO

The results of the experiments in these tests are summarised in the table on page 150
In no case is the bactericidal value of the compounds in vitro sufficiently great to account for the high curative power as shown by compounds such as p-aminobenzenesulphonamide and/

and M. & B.693 against haemolytic streptococcal infections of mice. Compounds such as acriflavine it will be recalled exhibit a bactericidal action at a dilution of about 1 in 500,000 and yet show little curative power in experimental infections.

Some compounds such as p-aminobenzene-sulphonamide coupled with p-hydroxybenzenesulphonamide and the two quinoline derivatives show a definite bacteriostatic effect against the haemolytic streptococcus, and yet, they have no curative action on a haemolytic streptococcal septicaemia in mice. The quinoline derivatives have also a slight bacteriostatic effect on pneumococcal, staphylococcal and Bacillus Aertrycke cultures, though neither compound has any effect in vivo against these organisms. This bacteriostatic effect is probably related to the presence in the molecule of a phenolic hydroxyl group and probably has no connection with the sulphonamide grouping. The bacteriostatic effect of 4:4'diacetyldiaminodiphenylsulphide on the staphylococcus and on BacillusnAertrycke is of definite interest. A slight parallelism can here be drawn between the in vivo and the in vitro results as 4:4'diacetyldiaminodiphenylsulphide has a definite curative effect on mouse staphylococcal septicaemia, but this compound has no action on a Bacillus Aertrycke infection

in/

in mice, though there is an effect in vitro. One of the relatively active antistreptococcal compounds in the animal experiments was 3-nitro-4-hydroxybenzene-sulphonamide and this compound also had a antibacterial effect for the haemolytic streptococcus in the test tube. The failure of compounds such as M. & B. 693, 1:4'di(p-aminobenzenesulphonyl)piperazine, and 1:4 di(p-acetylaminobenzenesulphonyl) piperazine, to show antistreptococcal activity in the test tube is rather surprising in view of their curative powers in the experimental animal. It can be seen that there is no very clear cut parallelism between Table I and Table II, and yet there are certain points of similarity such as those just mentioned above. The failure to find any parallelism is not inconsistent with the view that the bacteriostatic action of these compounds is connected with the mechanism of their action. This question will be discussed more fully in the next section.

MECHANISM OF ACTION

There are at the moment three explanations which are probable factors in the mode of chemo-therapeutic action of the sulphanilamide compounds. The first of these describes the mechanism as the purely bactericidal action of the drug on the bacteria. It is conceivable that these drugs act as 'internal antiseptics' in/

in the body and that there is a direct lethal action of the organisms in the blood and tissue, and though prontosil does not possess antibacterial powers in vitro, it has been shown that the blood of patients treated with it possesses an increased lethal action towards the streptococci. The relatively high killing power of sulphanilamide in blood as compared with water (Buttle et al, 1937) explains in part the high bactericidal power of sulphanilamide in the blood of experimental animals. However, in vitro experiments do not give sufficient support for a purely bactericidal explanation and there certainly must be some other factor concerned.

The second view, that neither prontosil nor p-aminobenzenesulphonamide act as such in the animal body, but are converted to some very much more active substance has been advocated. Such a theory would explain differences between the in vivo and the in vitro effects, and would be consistent with the variation shown by different animals in their susceptibility to the curative action of the compounds. This hypothetical intermediate compound was at one time thought to be a stage in the oxidation of sulphanilamide in the body. Domagk thought that the active agent was possibly p-nitrobenzenesulphonamide whereas Mayer thought the hydroxylamino derivative/

derivative of sulphanilamide was responsible for its high curative action in vivo.

According to the third theory sulphanilamide and related compounds produce an action on the defence mechanism of the bacteria of such a type that they are readily attacked by the leucocytes and possibly other elements of the defence mechanism of the body. This theory is not inconsistent with the second view as an intermediate compound might be the active agent. This is almost certainly the case with the original prontosil dyestuff which has no action in vitro and is reduced in the body to sulphanilamide which is active. However, the distinctive feature of this theory is the important role assigned to the leucocytes in actively attacking the bacteria maimed but not killed by the chemotherapeutic agent.

As suggested above there are strong reasons for believing that the sulphonamide derivatives can act simply in virtue of their bactericidal effect (Theory I) though the failure to obtain any close parallelism between in vitro and in vivo results may be regarded as evidence against this theory. There did, however, seem to be the possibility that although the compounds exerted relatively weak bactericidal action under the ordinary conditions employed in the test tube, a modification of certain factors might result in a great intensification of activity.

It is well known that the bactericidal action of compounds such as phenol is influenced by the pH of the medium. It was possible, therefore, that the modification of the pH and Eh of the environment might markedly modify the bactericidal action of sulphanilamide. The experiments recorded on pages 236 - 247 showed that this does not occur. This would seem to decrease the probability that the direct action of the compounds in the body is substantially intensified as compared with that observed under conditions of testing in vitro.

With regard to the second theory there is an absence of conclusive evidence in favour of any particular intermediate compound increasing the activity. The failure of the catalysed oxidation experiments described on pages 248 - 250 to give positive results is also against the second explanation though of course the possibility exists that under conditions not yet investigated the hypothetical intermediate compound might be produced.

We come then to the third of above mentioned theories, and it may be said at once that while the work described here was in progress a considerable amount of evidence has been published which gives strong support to this explanation, in particular we may refer to the work of Fleming (1938) who described/

in vitro experiments on the action of M. & B. 693 on pneumococci and streptococci. Fleming found that M. & B. 693 retarded the growth of these organisms in human blood and that leucocytes were necessary for bacterial destruction.

The results which are presented in this thesis although not affording any positive proof of this theory are consistent with it. Thus, it is easy to understand why negative results were obtained in experiments with pH and Eh and on catalysed oxidations. Furthermore the absence of any vigorous parallelism between the in vivo and the in vitro experiments is also not surprising.

Whitby (1938) found that after treatment with M. & B. 693 pneumococci exhibited degenerative features such as destruction of the bacterial capsules. Telling and Oliver (1938) believe they have confirmed this result. However, Fleming (1938) and Long and Bliss (1939) have been unable to see specific changes in the capsules of the pneumococci. Evidence of damaged forms of organisms is of course in favour of the above theory of the mechanism of action. It may be added that certain suggestions put forward that sulphonamide compounds have some other favourable action/

action in the infected animal body such as the stimulation of the leucocytes or neutralisation of bacterial toxins. These effects of course are not inconsistent with the third theory of mechanism of action and would obviously help in promoting it. However, it may be said that the evidence, at least in the case of toxin neutralisation, is somewhat conflicting.

. Thus, the evidence at present available would seem to jus tify us in concluding that sulphanilamide, M. & B. 693 and presumably other related sulphur containing compounds, exercise their curative action on the animal body by damaging the infecting bacteria in such a way as to restrict their multiplication and damage their protective mechanism against the leucocytes and other defence factors in the animal body, at the same time the compounds apparently exert no appreciable toxic effects on the leucocytes and may even stimulate them to increased activity. The result is that these natural defence mechanisms of the animal can deal effectively with the infection and bring about cure.

SUMMARY AND CONCLUSIONS

1. From the 20 compounds tested for their chemotherapeutic action against mice infected with the haemolytic streptococcus, p-aminobenzenesulphonamide, M. & B. 693, (2-sulphanilylaminopyridine), 3-nitro-4-hydroxybenzenesulphonamide, 3-nitro-4-aminobenzenesulphonamide, 1:4di(aminobenzenesulphonyl)piperazine and 1:4 di(acetylaminobenzenesulphonyl)piperazine showed definite activity. Of these 6 compounds, p-aminobenzenesulphonamide and 2-sulphanilylaminopyridine are already known to have antistreptococcal properties while 3-nitro-4-aminobenzenesulphonamide has been reported inactive. Investigations in the curative properties of the two piperazine derivatives, and of 3-nitro-4-hydroxybenzenesulphonamide have not been before reported.

2. Against a pneumococcal infection in mice p-aminobenzenesulphonamide had a slight death delaying effect, but the other 12 compounds examined were inactive.

3. Using the staphylococcus as the infecting organism, diacetyldiaminodiphenylsulphide and 1:4 di(acetylaminosulphonyl)piperazine had/

had a protective action on the infected mice, while M. & B. 693 (2-sulphanilylaminopyridine) delayed death in the animals injected with the staphylococcus and fed with this compound.

4. No protective power could be demonstrated with any of the 20 compounds against mice infected with Bacillus Aertrycke.

5. With some of the compounds a bactericidal effect was evident in vitro against the streptococcus and the pneumococcus, but against the staphylococcus and Bacillus Aertrycke there was little effect from any of the compounds. The results of the in vitro experiments do not run parallel to those obtained in the animal experiments, thus the effect in the animal body must be other than a simple bactericidal one.

6. The results are against the suggestion that the special conditions of animal tissue in respect of pH and Eh might modify the bactericidal action observed in the in vitro results.

7. No evidence could be obtained, from attempts to oxidise sulphanilamide by atmospheric oxygen/

oxygen in presence of enzymes or of manganese salts, that a highly active oxidation product could be formed.

8. The bearing of these results on the mechanism of the action of sulphanilamide is discussed.

I should like to express my very grateful thanks to Dr W.O. Kermack for all his help and encouragement in the course of this work. I should also like to thank the Superintendent of the Laboratory of the Royal College of Physicians for granting me facilities to work in Edinburgh, and Professor W.J. Tulloch for hospitality in Dundee.

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