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THE CHEMOTHERAPY OF *CL. WELCHII* TYPE A AND *CL. SEPTIQUE* IN MICE

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Although it would appear that the value of drugs of the sulphanilamide group in the treatment of wound infections has not yet been extensively studied, the suggestion from the War Office (1939) "that all wounds which from the clinical aspect appear likely to become the site of secondary coccal or gas-gangrene infection should receive a prophylactic course of sulphanilamide treatment" has prompted the publication of these preliminary results. In the provisional memorandum quoted above it is emphasized that, although the effectiveness of the drugs in the treatment of streptococcal infections is proved, there are only indications that they also have an action on anaerobic gas-forming bacilli. Long and Bliss (1937) reported the first experimental results of sulphanilamide therapy in *Clostridium welchii* infections. Using an intraperitoneal infection with spun cultures resuspended in broth, they were able to save 70 per cent. of mice treated with sulphanilamide, whereas 94 per cent. of the controls died. Domagk (1937) found that uleron was effective in experimental gas-gangrene infection (Fraenkel strain). This compound was considered more effective than sulphanilamide, but no comparative data were given. Bohlman (1937) treated three clinical cases of gas gangrene, following crushing injuries, with sulphanilamide. All three cases had received a prophylactic injection of "combined gas-gangrene antitoxin," but this treatment failed to prevent the development of the disease. They all responded promptly to sulphanilamide, however, and these results were later confirmed by Kennedy (1938).

Our experiments were designed to throw some light on the possible influence of drugs of the sulphanilamide group on infections with *Cl. welchii* Type A (*B. perfringens*) and *Cl. septique* in mice. In preliminary experiments it was found that these drugs were unable to prevent the death of mice injected intravenously with approximately one minimum lethal dose of the toxin of either of these organisms.

CLOSTRIDIUM WELCHII TYPE A

The outcome of experimental infections with *Cl. welchii* in mice varies considerably according to the strain employed, the number of organisms injected, and the route

of infection. As is well recognized, strains may show considerable variation in virulence or invasiveness and also in ability to produce toxin. While we have carried out preliminary observations with several strains, two were chosen for more extended work. One, "Corcoran," isolated recently from a case of gas gangrene in man, was known to be a poor toxin producer; the other, "S.R.12," isolated twenty years ago from a similar source, has long been employed for the production of toxin in these laboratories, and is an excellent toxigenic strain. With a view to simulating natural wound infection we adopted a suggestion, received in a personal communication from Colonel L. E. H. Whitby, R.A.M.C., that the organisms be injected in a suspension of sterile soil. In each experiment a three-hour culture in 250 c.cm. of meat broth was used, the opacity of the supernatant corresponding fairly consistently with tube No. 7 of the "Wellcome" standard opacity series.

No spores were seen in smear preparations, but only vegetative forms, although samples from some of the cultures resisted heating to 80° C. for fifteen minutes and therefore presumably contained small numbers of spore forms. The supernatant of each culture was centrifuged and the deposit resuspended in varying amounts of broth, the broth suspensions being then diluted in sterile 10 per cent. suspensions of soil in saline. Mice of 24 to 26 grammes were injected either intraperitoneally or intramuscularly. The drugs sulphapyridine (M & B 693) and sulphanilamide were suspended in distilled water after grinding in gum acacia, and were given by stomach tube to groups of mice, which were then observed for at least seven days. A number of experiments gave similar results, and only typical protocols are therefore quoted.

Strain "Corcoran" Injected Intraperitoneally

In preliminary experiments, on sixty mice in groups of six, courses of treatment with either sulphapyridine or sulphanilamide were started one hour before infection, and were continued for four, nine, twenty-four, and forty-eight hours afterwards. The drugs appeared to be equally effective, the usual result being that doses ranging from 25 mg. to 1 mg. prevented the death of all the mice

injected intraperitoneally with approximately five fatal doses of infected soil suspension. Doses of 0.2 mg. of either drug saved only 50 per cent. of the animals. All infected control mice died overnight, and controls injected with uninfected soil suspensions survived. In another experiment, in which approximately twenty fatal doses were injected, the drugs failed to hold up infection except in the case of sulphapyridine, repeated doses of 50 mg. of which saved four of six mice. It was subsequently found that a single dose of either sulphapyridine or sulphanilamide was effective against five fatal doses, provided the drugs were given either before or an hour or so after infection (Tables I and II). "Delayed treatment" with sulphapyridine was possibly slightly more effective than that with sulphanilamide. Treatment with antitoxic serum was a failure.

As in clinical practice it may not always be possible to administer the drugs by mouth an experiment was carried out with soluble forms. Sulphapyridine soluble in doses of 5 mg. or 1 mg., or sulphanilamide in solution

TABLE I.—*Mice Injected Intraperitoneally with Strain "Corcoran" and Treated with Sulphapyridine in Comparison with Serum*

Infection.—Spun deposit from 3-hour culture resuspended in half the original volume of broth. Broth suspension diluted 1 in 20 in soil suspension, so that final dilution of culture was 1 in 10. 0.5 c.cm. (approximately 5 fatal doses) injected intraperitoneally.

Drug Treatment.—Groups of 6 mice given a single specified dose at varying intervals from the time of infection.

Serum Treatment.—Groups of 6 mice given a single specified dose of *Cl. welchii* Type A antitoxin (500 international units per c.cm.) subcutaneously or intravenously.

Controls.—(a) 11 infected mice, untreated; (b) 2 mice injected with 0.5 c.cm. of sterile soil suspension.

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead Later	Living 10 Days
I	50 mg. 1 hour before infection ..	0	0	0	6
II	25 " " " " " ..	0	0	1	5
III	5 " " " " " ..	0	0	2	4
IV	1 " " " " " ..	1	2	0	3
V	50 " at time of infection ..	1	1	0	4
VI	25 " " " " " ..	0	0	0	6
VII	5 " " " " " ..	0	1	0	5
VIII	1 " " " " " ..	2	2	0	2
IX	50 " 1 hour after infection ..	0	1	0	5
X	25 " " " " " ..	0	0	0	6
XI	5 " " " " " ..	2	1	0	3
XII	1 " " " " " ..	0	4	0	2
XIII	50 " 2 hours " " ..	0	4	1	1
XIV	25 " " " " " ..	1	0	2	3
XV	5 " " " " " ..	0	0	0	6
XVI	1 " " " " " ..	1	1	4	0
XVII	50 " 3 hours " " ..	5	1	0	0
XVIII	25 " " " " " ..	4	2	0	0
XIX	5 " " " " " ..	2	3	0	1
XX	1 " " " " " ..	3	2	1	0
XXI	0.4 c.cm. serum s/c 1 hr. before infection	3	3	0	0
XXII	0.4 " " " " " after " "	5	1	0	0
XXIII	0.4 " " " i/v " before " "	3	2	1	0
XXIV	0.4 " " " " " after " "	5	1	0	0
XXV	Infected controls ..	9	1	0	1
XXVI	Soil-injected controls ..	1*	0	0	1

* Due to intercurrent disease (parasitic worm cyst in liver). In other experiments larger numbers of soil-injected controls were used. This death was one of two among 72 mice so injected.

TABLE II.—*Mice Injected Intraperitoneally with Strain "Corcoran" and Treated with Sulphanilamide in Comparison with Serum. Procedure as in Table I, but only 6 Infected Mice, Untreated, kept as Controls*

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead Later	Living 7 Days
I	25 mg. 1 hour before infection ..	0	0	0	6
II	5 " " " " " ..	0	0	0	6
III	1 " " " " " ..	3	2	0	1
IV	25 " at time of infection ..	0	0	0	6
V	5 " " " " " ..	1	1	1	3
VI	1 " " " " " ..	3	0	0	3
VII	25 " 1 hour after infection ..	1	0	1	4
VIII	5 " " " " " ..	0	1	0	5
IX	1 " " " " " ..	2	4	0	0
X	25 " 2 hours " " ..	5	0	0	1
XI	5 " " " " " ..	2	3	0	1
XII	1 " " " " " ..	2	2	2	0
XIII	0.4 c.cm. serum s/c 24 hrs. before infection	1	5	0	0
XIV	0.4 " " " i/v " " "	0	4	2	0
XV	0.4 " " " 3 hrs. " " "	2	4	0	0
XVI	Infected controls ..	5	1	0	0
XVII	Soil-injected controls ..	0	0	0	2

in similar doses, was given orally or subcutaneously to groups of mice one hour before and four hours after infection with approximately five fatal doses of strain "Corcoran." Nearly all the mice thus treated survived, indicating that the drugs are active in solution when given either orally or subcutaneously.

Strain "Corcoran" Injected Intramuscularly

There is some contrast between the results in the foregoing experiments and those in which infection was by the intramuscular route. The experiment shown in Table III is typical of several: even with the greater number of organisms injected there was seldom more than a 50 per cent. mortality in the untreated mice, although local reactions at the site of injection of the infecting doses were severe. The drugs appeared to exert no inhibitory action, although serum regularly prevented the appearance of anything more than a slight local reaction.

TABLE III.—*Mice Injected Intramuscularly with Strain "Corcoran" and Treated with Sulphapyridine, Sulphanilamide, or Serum. Groups of 6 Mice Throughout*

Infection.—Spun deposit from 3-hour culture resuspended in one-tenth of the original volume of broth. Broth suspension diluted 1 in 2 in soil suspension, so that final concentration of culture was fivefold. 0.25 c.cm. injected into the muscles of the leg.

Drug Treatment.—25 mg. 1 hour before and 4, 9, 24, and 48 hours after infection.

Serum Treatment.—0.4 c.cm. of *Cl. welchii* Type A antitoxin (750 international units per c.cm.) intravenously 1 hour before infection.

Controls.—(a) Infected mice, untreated; (b) mice injected with 0.25 c.cm. of sterile soil suspension.

Group		Dead	Survived 7 Days but Local Reactions			
			Severe	Moderate	Slight	Nil
I	Sulphapyridine ..	5	0	1	0	0
II	Sulphanilamide ..	1	0	5	0	0
III	Serum ..	0	0	0	6	0
IV	Infected controls ..	3	3	0	0	0
V	Soil-injected controls ..	0	0	0	2	4

In other experiments the infecting dose was reduced so as to be sublethal, the concentration of organisms being such that the untreated mice showed moderately severe local reactions. Treatment with either drugs or serum now produced a definite effect, the local reactions at the sites of injection of the infecting dose being very slight or absent. The dose necessary to produce any significant swelling intramuscularly was equivalent to approximately five fatal doses when injected intraperitoneally. The intramuscular dose required to produce a 50 per cent. mortality was equivalent to approximately 100 fatal doses when injected intraperitoneally.

Strain "S.R.12" Injected Intraperitoneally

As has already been stated, "S.R.12" is a highly toxigenic strain used regularly for the production of toxin in these laboratories. In a typical experiment (Table IV) sulphapyridine or sulphanilamide given one hour before and continued up to the forty-eighth hour after infection prevented the death of the majority of mice injected intraperitoneally with approximately ten fatal doses of this strain. Serum given intravenously one hour before infection saved four out of six mice similarly injected.

TABLE IV.—*Mice Injected Intraperitoneally with Strain "S.R.12" and Treated with Sulphapyridine, Sulphanilamide, or Serum. Groups of 6 Mice Throughout*

Infection.—Spun deposit from 3-hour culture resuspended in half the original volume of broth. Broth suspension diluted 1 in 50 in soil suspension, so that final dilution of culture was 1 in 25. 0.5 c.cm. (approximately 10 fatal doses) injected intraperitoneally.

Drug Treatment.—Sulphapyridine or sulphanilamide in the specified amount 1 hour before and 4, 9, and 24 hours after infection.

Serum Treatment.—0.4 c.cm. of *Cl. welchii* Type A antitoxin (900 international units per c.cm.) intravenously 1 hour before infection.

Controls.—(a) Infected mice, untreated; (b) mice injected with 0.5 c.cm. of sterile soil suspension.

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead Later	Living 10 Days
I	25 mg. sulphapyridine ..	0	1	0	5
II	5 " " ..	0	0	0	6
III	1 " " ..	0	1	2	3
IV	0.2 " " ..	1	0	3	2
V	25 " sulphanilamide ..	0*	1	2	3
VI	5 " " ..	1	0	1	4
VII	1 " " ..	0	0	0	6
VIII	0.2 " " ..	3	0	0	3
IX	0.4 c.cm. serum ..	0	1	1	4
X	Infected controls ..	6	0	0	0
XI	Soil-injected controls ..	0	0	0	6

* 1 mouse showed toxic symptoms.

In another experiment where a greater number of organisms were used and approximately twenty-five fatal doses were injected, serum again gave almost complete protection, whereas there were several deaths in the drug-treated groups.

Strain "S.R.12" Injected Intramuscularly

This strain is much more toxigenic than strain "Corcoran"; 0.25 c.cm. of a fivefold concentration of the culture injected intramuscularly was equivalent to approximately fifty fatal doses, whereas with 0.25 c.cm. of a fivefold concentration of culture of strain "Corcoran" only 50 per cent. of mice died, corresponding to approximately one fatal dose (see Table III). Drug treatment was quite unable to save the mice infected with fifty fatal doses of "S.R.12," 90 per cent. of the deaths occurring within nine

hours. Antitoxic serum injected one hour before infection saved all the mice, although each showed slight swelling at the site of infection, which persisted for two to three days and then quickly resolved. None of the soil-injected controls showed any swelling. When the number of fatal doses injected was reduced to ten similar results were still obtained. In other experiments subfatal doses produced moderately severe local reactions in the control mice, but those treated with either of the drugs showed distinctly less swelling, and those treated with serum had no local reactions. It is of interest that many more organisms were necessary to cause the death of the mice by intramuscular injection than by intraperitoneal: one fatal dose injected intramuscularly was equivalent to approximately ten fatal doses when injected intraperitoneally.

With three other strains of *Cl. welchii* Type A, preliminary experiments indicated that the drugs were of some therapeutic value.

Discussion on Experiments in *Cl. welchii* Infections

The experiments with these two strains demonstrate that sulphapyridine and sulphanilamide are of therapeutic value in *Cl. welchii* Type A infections in mice. Even a single moderate dose of either drug given one hour after intraperitoneal infection with a few fatal doses usually prevented death; sulphapyridine may be somewhat more effective than sulphanilamide. When infection was by the intramuscular route it was more difficult to demonstrate any influence of drug treatment. Only when a sublethal infecting dose was used did it have a favourable effect. There appeared, then, to be some variation in efficiency according to the route of infection. Since, however, the number of organisms in the few fatal doses in intraperitoneal infections was very similar to that in the dose used in the sublethal intramuscular infections—in both of which the drugs were efficient—it would seem that the number of infecting organisms which can be dealt with by the drugs does not vary much with the route of infection.

The two strains principally used differed in their capacity to produce toxin, "S.R.12" producing a toxin approximately three times more potent than that of the "Corcoran" strain. Even when the dose of culture was adjusted to avoid death within nine hours the drugs appeared slightly less effective against the more toxic strain.

Mice infected intraperitoneally with the "S.R.12" strain appeared to be more susceptible to the toxic effects of sulphanilamide than were normal mice. When repeated doses of 25 mg. of this drug were given to mice of 21 to 23 grammes toxic symptoms were observed on several occasions. This was not so apparent in intraperitoneal infections with the "Corcoran" strain, nor were any toxic symptoms observed with either strain when full and repeated doses of sulphapyridine were employed. In order to avoid these effects mice between 24 and 26 grammes were used for experiments in which large doses of sulphanilamide were to be administered to certain groups.

Although serum was employed only for comparative purposes a particularly interesting observation was made. Serum failed to prevent death following intraperitoneal injection of the "Corcoran" strain, whereas it generally gave complete protection against intraperitoneal injection of the more toxigenic "S.R.12" strain and also against intramuscular injection of either strain. Five samples of serum used gave the same result. All had been prepared

by immunization of horses with the toxin of the "S.R.12" strain, and in *in vivo* titrations each neutralized the toxins produced by both the "Corcoran" and the "S.R.12" strains. It may be that the sera contained some strain-specific antibody which prevented invasion by "S.R.12" but not by "Corcoran" when infection was by the intraperitoneal route. Alternatively, the failure of the serum to protect mice injected intraperitoneally with the "Corcoran" strain suggests that the latter may be more invasive than the "S.R.12" strain. This supposition is borne out by the fact that pure cultures of *Cl. welchii* were recovered from the heart blood of forty-five out of forty-eight mice infected with the "Corcoran" strain and from only thirty-four out of sixty-one of those infected with the "S.R.12" strain. Further work on this problem is in progress.

An attempt has been made to summarize the results of the experiments with *Cl. welchii* (Table V), but it should be noted that a single dose of serum is compared with one dose of drug in some experiments and with multiple doses in others, and that the times of injection are also different. A summary expressing results obtained under the conditions adopted for the series of experiments may, however, be of some use.

TABLE V.—The Relative Value of Drugs and Serum in *Cl. welchii* Infection in Mice

Strain	Route of Infection	Approx. No. of Fatal Doses in the Infecting Dose	Relative No. of Organisms in the Infecting Dose	Results of Treatment	
				Drugs	Serum
"Corcoran"	Intraperitoneal	20	100 N	Slight*	—
"	"	5	25 N	Very good	Nil
"	Intramuscular	1	625 N	Nil	Good
"	"	<1	25 N	Good	"
"S.R.12"	Intraperitoneal	25	25 N	Fairly good	"
"	"	10	10 N	Good	"
"	Intramuscular	50	625 N	Nil	"
"	"	10	125 N	"	"
"	"	<1	12.5 N	Good	V. good

* Only with large doses of sulphapyridine.

The number of organisms injected in these experiments was not ascertained, but, since a standard method of cultivation was employed throughout, it is reasonable to assume that there was no great variation in the density of the cultures used. On this basis one may express the infecting doses in terms of numbers of organisms by assigning the letter N to the number contained in the smallest amount of "S.R.12" culture which caused a fatal infection when injected by the intraperitoneal route. This has been done in the above table.

CLOSTRIDIUM SEPTIQUE

Two strains of *Cl. septique* were employed. One, a Type I strain, "Pasteur," was used as a spore suspension in 1 per cent. calcium chloride solution; the other, a Type III strain, "V.S.20," was used as a suspension of the washed organisms (spores and vegetative forms) from a twenty-four-hour culture, and was injected in sterile soil suspension. Both strains produce a potent toxin and cause a rapidly fatal infection by either the intraperitoneal or the intramuscular route.

Strains "Pasteur" and "V.S.20" Injected Intramuscularly

While our observations with *Cl. septique* have not been so extensive as those with *Cl. welchii* the experiments quoted (Tables VI, VII, and VIII) are typical of several others. Drug treatment was along lines similar to those detailed earlier.

TABLE VI.—Mice Injected Intramuscularly with Type I Strain, "Pasteur," and Treated with Sulphapyridine or Sulphanilamide. Groups of 6 Mice Throughout

Infection.—A suspension of spores in 1 per cent. CaCl_2 solution. 0.5 c.cm. (estimated number of spores approximately 50,000) injected into the muscles of the leg.

Drug Treatment.—Sulphapyridine or sulphanilamide. 40 mg. of each drug given orally either half an hour before infection or immediately after, as specified; further doses given 6, 24, and 48 hours after infection.

Controls.—(a) Infected mice, untreated; (b) mice injected with 0.5 c.cm. of 1 per cent. CaCl_2 solution.

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead 72 Hrs.	Dead Later	Living 9 Days
I	Sulphapyridine $\frac{1}{2}$ hour before infection ..	0	2	0	1	3
II	Sulphapyridine immed. after infection ..	0	4	0	2	0
III	Sulphanilamide $\frac{1}{2}$ hour before infection ..	1	4	0	1	0
IV	Sulphanilamide immed. after infection ..	3	2	0	1	0
V	Infected controls ..	6	0	0	0	0
VI	CaCl_2 injected controls ..	0	0	0	0	6

Sulphanilamide treatment of mice injected intramuscularly with this strain was of little value, whereas treatment with sulphapyridine begun either half an hour before or immediately after infection delayed the death of all the mice for twenty-four hours, and when begun half an hour before infection was able to save three of six infected mice.

TABLE VII.—Mice Injected Intramuscularly with Type I Strain, "Pasteur," and Treated with Sulphapyridine in Comparison with Serum. Groups of 6 Mice Throughout

Infection.—A suspension of spores in 1 per cent. CaCl_2 solution. 0.5 c.cm. (estimated number of spores approximately 200) injected into the muscles of the leg.

Drug Treatment.—40 mg. sulphapyridine orally, either half an hour before or immediately after infection, as specified.

Serum Treatment.—0.3 c.cm. of *Cl. septique* antitoxin (600 international units per c.cm.) intravenously at a specified interval after infection.

Combined Drug and Serum Treatment.—40 mg. sulphapyridine orally immediately after infection. 0.3 c.cm. of the above serum injected intravenously at a specified interval after infection.

Controls.—(a) Infected mice, untreated; (b) mice injected with 0.5 c.cm. of 1 per cent. CaCl_2 solution.

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead 72 Hrs.	Dead Later	Living 14 Days
I	Sulphapyridine $\frac{1}{2}$ hour before infection ..	0	1	3	0	2
II	Sulphapyridine immed. after infection ..	0	3	1	2	0
III	Serum 6 hours after infection ..	0	1	2	1	2
IV	Serum 12 hours after infection ..	0	1	1	2	2
V	(Sulphapyridine immed. after infection Serum 6 hours after infection ..)	1	0	0	1	4
VI	(Sulphapyridine immed. after infection Serum 12 hours after infection ..)	0	0	1	1	4
VII	(Sulphapyridine immed. after infection Serum 24 hours after infection ..)	0	0	0	1	5
VIII	Infected controls ..	6	0	0	0	0
IX	CaCl_2 injected controls ..	0	0	0	0	6

It will be seen (Table VII) that serum treatment of mice injected intramuscularly with this strain, even when delayed for twelve hours after infection, was as effective as sulphapyridine given half an hour before infection. The best results were obtained by combining treatment with sulphapyridine and serum, the drug being given immediately after and the serum up to twenty-four hours after infection.

TABLE VIII.—*Mice Injected Intramuscularly with Type III Strain, "V.S.20," and Treated with Sulphapyridine or Sulphanilamide. Groups of 6 Mice Throughout*

Infection.—Spun deposit from 24-hour culture resuspended in one-twentieth of the original volume of broth. Broth suspensions (opacity four times greater than "Wellcome" standard opacity tube No. 10) diluted 1 in 100 in soil suspension. Final dilution of culture was 1 in 5. 0.25 c.cm. (approximately 100 fatal doses) injected into the muscles of the leg.

Drug Treatment.—Sulphapyridine or sulphanilamide in a specified amount 1 hour before infection, 4 and 9 hours after, and thereafter twice daily for two days and once daily for three days.

Serum Treatment.—*Cl. septicum* antitoxin (650 international units per c.cm.) intravenously.

Controls.—(a) Infected mice, untreated; (b) mice injected with 0.5 c.cm. of sterile soil suspension.

Group		Dead 24 Hrs.	Dead 48 Hrs.	Dead 72 Hrs.	Dead Later	Living 7 Days
I	50 mg. sulphapyridine ..	0	0	0	2	4
II	25 " " ..	0	1	1	2	2
III	5 " " ..	0	4	2	0	0
IV	25 " sulphanilamide ..	0	5	0	1	0
V	5 " " ..	0	5	0	1	0
VI	0.4 c.cm. serum 1 hour before infection ..	0	2	0	1	3
VII	0.4 c.cm. serum 1 hour after infection ..	0	2	1	1	2
VIII	Infected controls ..	5	1	0	0	0
IX	Soil-injected controls ..	0	0	0	0	6

Table VIII shows that sulphanilamide treatment of mice injected intramuscularly with approximately 100 fatal doses of this strain delayed the death of the mice for twenty-four hours. All mice treated with sulphapyridine survived for twenty-four hours, and with the higher doses there were some survivals seven days later. A single dose of serum either before or after infection was as effective as repeated doses of sulphapyridine. In another experiment, in which the infecting dose was approximately ten fatal doses, treatment with the drugs gave very similar results; whereas when 1,000 fatal doses were used the drugs had no influence, the control and treated mice dying within nine hours.

Strain "V.S.20" Injected Intraperitoneally

In a number of experiments similar to that shown in Table VIII the infecting dose was injected intraperitoneally. As with *Cl. welchii*, in intraperitoneal infections fewer organisms were required to produce deaths than in intramuscular infections. In contrast, however, the results of drug treatment of *Cl. septicum* infections did not vary markedly according to the route of infection. In intraperitoneal infections it was possible to delay death of all the mice and, in the case of sulphapyridine, to save some of the mice when 100 or 1,000 fatal doses were injected, but if the number of fatal doses was increased to 10,000 the effect of drug treatment was almost negligible, and with 100,000 fatal doses no effect was observed. A single dose of serum was as effective as repeated doses of sulphapyridine against 100 or 1,000 fatal doses of culture.

Discussion on *Cl. septicum* Experiments

It is evident that sulphanilamide does little more than delay death for twenty-four hours. Sulphapyridine delays death for at least twenty-four hours, and in groups of infected mice treated with large doses there may be some survivals. Treatment with antitoxic serum, even when delayed for twelve hours, was as effective as drug treatment with sulphapyridine given from the start of the infection. The best effect was obtained when a large dose of sulphapyridine was given immediately after

infection and was followed by serum given up to twenty-four hours later. The results of the experiments with *Cl. septicum* have been summarized in the following table.

TABLE IX.—*Relative Value of Drugs and Serum in Cl. septicum Infections in Mice*

Strain	Route of Infection	Approx. No. of Fatal Doses in Infecting Dose	Approx. No. of Spores in Infecting Dose	Results of Treatment		
				Sulphapyridine	Sulphanilamide	Serum
" Pasteur "	Intramuscular		50,000	Fairly good	Slight	—
" "	"		200	"	—	Fairly good
" "	"		200	Sulphapyridine and serum combined—Good		
			Relative No. of Organisms in Infecting Dose			
" V.S.20 "	"	1,000	500,000 N	Nil	Nil	—
" "	"	100	50,000 N	Fairly good	Slight	Fairly good
" "	"	10	5,000 N	"	"	—
" "	Intraperitoneal	100,000	100,000 N	Nil	Nil	—
" "	"	10,000	10,000 N	Very slight	"	—
" "	"	1,000	1,000 N	Fairly good	Slight	Fairly good
" "	"	100	100 N	"	"	"

The letter N represents the smallest number of organisms which caused a fatal infection when injected by the intraperitoneal route.

Summary of Conclusions

Cl. welchii Type A.—Sulphanilamide and sulphapyridine protected mice against a small number of lethal doses injected intraperitoneally as suspensions of vegetative organisms in sterile soil suspension. Treatment with antitoxic serum was also effective in the case of a strain of high toxigenicity, but failed against a strain of low toxigenicity but probably of higher invasiveness. When the infected soil suspensions were injected intramuscularly considerably more organisms were necessary to produce a fatal result and the drugs were of value only against a sublethal infection. Serum treatment was better, and saved mice even from lethal doses.

Cl. septicum.—Sulphanilamide had little influence on infections of mice injected intraperitoneally or intramuscularly with suspensions of sporing and non-sporing organisms in calcium chloride solution or sterile soil suspension. Sulphapyridine was better: in large doses it saved 50 per cent. of the animals. Treatment with a single dose of antitoxic serum was at least as effective as sulphapyridine, but the best results were obtained when sulphapyridine was combined with serum, large doses of the drug being given immediately after infection and serum up to twenty-four hours later.

With two strains of *Cl. oedematiens* tested so far it has been shown that neither sulphanilamide nor sulphapyridine has any influence on the course of the infection.

Although much remains to be done these findings are published because they are of topical interest.

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