PREVENTION OF GAS-GANGRENE INFECTIONS IN EXPERIMENTAL WOUNDS BY LOCAL APPLICATION OF SULPHONAMIDE COMPOUNDS AND BY SERA

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This paper describes an experimental investigation into the possibility of using sulphonamide compounds to prevent the development of gas gangrene in wounds. Briefly, experimental wounds were made in guinea-pigs; one of the compounds was applied, a massive inoculation of *Cl. welchii*, *Cl. septicus*, or *Cl. oedematiens* was introduced, and then the wound was closed, the efficiency of the treatment being indicated by the survival or death of the animal. This procedure involves two main problems: the activity of these compounds against anaerobes, and the value of treatment by local application instead of general systemic administration.

Literature

(a) Activity of Sulphonamide Compounds against Anaerobes.

—The earliest reports on this subject appear to be those of Long and Bliss (1937) and Domagk (1937), who found that sulphanilamide and uleron would save mice from infection with *Cl. welchii*. Bohlman (1937) treated three cases of gas gangrene with sulphanilamide, and obtained a prompt response. More recently Stephenson and Ross (1940) have investigated the effect of these compounds in mice, the bacilli being injected intraperitoneally or intramuscularly and the drug being given orally. In the case of *Cl. welchii* they found that sulphanilamide and sulphapyridine would protect many of the animals. With *Cl. septicus* sulphanilamide had little action, but sulphapyridine in large doses saved 50% of the animals. With *Cl. oedematiens* neither sulphanilamide nor sulphapyridine had any effect. In most cases treatment with serum was better than treatment with drugs, but the best results were obtained by a combination of the two. Henderson and Gorer (1940) obtained roughly similar results. McIntosh (at a recent conference) reported that, in mice, sulphathiazole was the most active compound, whether given generally or locally; but infections with *Cl. oedematiens* were difficult to influence. Burton, McLeod, McLeod, and Mayr-Harting (1940) found that the anaerobes are relatively insensitive to sulphanilamide and sulphapyridine *in vitro*, but highly sensitive to *p*-nitrobenzene-sulphonamide. Fuller (personal communication) finds that *in vitro* the anaerobes are most sensitive to sulphathiazole, less to sulphapyridine, and least to sulphanilamide.

(b) Local Application of Sulphonamides to Wounds and Similar Lesions.—The earlier literature on this subject contains a number of clinical papers, often with little evidence in support of their statements—e.g., Jaeger (1936), Tiling (1936), Brown (1937), Merz (1937), Bosse and Bosse (1939), Childrey (1939), and Smith (1940). Much more convincing is the paper by Jensen, Johnsrud, and Nelson (1939). They treated 41 cases of compound fracture with 5 to 15 grammes of sulphanilamide applied locally, combined with the usual surgical measures. The infection rate had previously been about 27%, but in their series only two cases (5%) became infected—due in both cases to having been re-compounded. The concentration in serum cozing from the wounds was 250 to 660 mg. per 100 c.cm. (the maximum solubility being about 800 mg.). They found also that the local application of sulphanilamide promoted the healing of staphylococcal infections of experi-

mental fractures in guinea-pigs. Later papers have tended to confirm their findings. Thus Sézary (1939) reports that soft chancres and phagedenic ulcers heal rapidly if they are dressed with powdered sulphanilamide, and Riou (1939) confirms this, using a paste containing 10% sulphapyridine. Mayo and Miller (1940) have recommended a saturated solution of sulphanilamide in saline for the irrigation of wounds following resection of the rectum; and Herrell and Brown (1940) have obtained encouraging results by applying a mixture of 3 grammes of sulphanilamide in 100 ml. saline to a variety of wounds, including infected thoracic and abdominal cavities. Wardle (1940), Jeffrey (1940), and Eisenhammer (1940) have found that the insertion of sulphanilamide into the knee-joint cavity causes no reaction. Chorine (1940) obtained very good results when 31 wounds were dressed with sulphanilamide. Levaditi, Bréchet, Gérard-Moissonier, and Tournay (1940) applied sulphanilamide or prontosil soluble to 12 older wounds infected with streptococci: marked clinical improvement was obtained in every case, and 5 of the wounds became free from these organisms. Colebrook (1940b) has similarly found that the local application of sulphanilamide will remove streptococcal infections from wounds. During recent months the application of sulphonamide compounds to wounds has been discussed by Colebrook (1940a), Jeffrey (1940), Buxton (1940), and others; unfortunately the disturbed conditions of war have so far prevented the collection of a satisfactory series of treated cases, complete with adequate controls.

On the experimental side the local application of sulphonamides seems to have attracted little attention at first apart from an unsuccessful attempt by Nicholson (1938) to treat streptococcal empyema in rabbits by intrapleural injections of prontosil soluble. With the outbreak of war, work was started in France. Nitti (1939) made experimental wounds in the dorso-lumbar muscles of rabbits. He applied a small compress of gauze soaked in a culture of streptococci, and on top of this about 1 gramme of sulphanilamide, the skin being finally closed. The controls died in twenty-four to forty-eight hours. Treated animals were still alive forty-eight hours later; the wound was reopened, the gauze removed, and new sulphanilamide added; under these circumstances most of the treated rabbits survived. The local application of sulphanilamide to a wound never caused irritation, its toxicity was low, and it was all absorbed and eliminated in forty-eight to sixty hours. Legroux (1940) used a technique closely resembling that of the present paper (to which it served as a pattern). His main experiments were similar to those of Nitti. He also made studies in guinea-pigs, the wound being dusted with sulphanilamide and infected by the insertion of a piece of cloth soaked in a culture of *Cl. welchii* or *Cl. histolyticum*. Controls died in two to three days. Animals which had received sulphanilamide died after four to seven days; but if the wound was reopened, the cloth removed, and new sulphanilamide added they survived. Delay of the treatment for more than one hour after insertion of the culture or administration of the compound by mouth instead of by local application resulted in death of the guinea-pigs. In similar experiments with *Cl. tetani* sulphanilamide produced only a slight prolongation of life.

Experimental

The experimental technique used in the present work was based on that of Legroux (1940). Under full anaesthesia with chloroform, an incision was made on the inside of the thigh of a guinea-pig (weight 200 to 250 grammes). The muscles anterior and posterior to the femur were well crushed with Spencer Wells forceps, the main blood vessels being spared. The drug (0.2 gramme) was then spread over and into the crushed muscle. A piece of cloth 15 by 15 mm, soaked in the bacterial culture was placed on top of the muscle and the wound was tightly closed by continuous suture. After three days, in animals which survived, the wound was reopened under an anaesthetic, the cloth was removed, and fresh drug (0.2 gramme) was applied, the skin being resutured loosely. The culture used was a twenty-hour growth in meat broth containing glucose. The cloth took up about 0.1 gramme of the fluid. Numerous control experiments showed that even four times this volume of fluid, sterilized by filtration, did not contain enough toxin to kill a guinea-pig. The density of the culture was estimated roughly by comparing its capacity with that of standard Brown's tubes. In order to avoid error due to abnormal strains of organisms numerous different strains were examined. The origin of these is as follows.

Cl. welchii :

- A.5 : isolated May, 1940, from compound fracture ; no gangrene
S.R.12 : " 1915, " fatal gas gangrene
Corcoran : " 1939, " peritonitis
A.7 : " 1940, " non-fatal gas gangrene
S.107 : animal origin, used as toxin-producer at Marburg for many years (Henderson, personal communication).
Roshier : isolated, 1940, from puerperal gas gangrene
Hudson : " June, 1940, from wound without gas gangrene
Barnetson : " " " wound of chest ; empyema
Constantine : " " " non-fatal gas gangrene

Cl. septicum :

- V.S.14 : Pasteur III, from Nat. Coll. type cultures—Type I
V.S.20 : isolated 1914–18—Type III
V.S.A.48 : " June, 1940, from non-fatal case of tetanus
V.S.A.52 : " " " osteomyelitis and ? gas gangrene

Cl. oedematiens :

- No. 3 : from Nat. Coll. type cultures (140, No. 3908). More than 11 years old
Cossard : ditto, isolated, 1918, from fatal gas gangrene

In most of the experiments the control untreated animals died in twenty-four hours, and showed the typical appearances of purplish subcutaneous oedema spreading out from the wounded thigh and involving much of the abdomen and thorax. Bacilli were present in the oedema fluid, in the peritoneum, and often in the heart blood taken shortly before death. Treated animals which died in the first two days showed post-mortem appearances similar to the controls. In a few cases in which the invasiveness of the organisms and the restraining influences (defence mechanisms plus drug) were approximately balanced a spread of gangrenous oedema took place over part of the abdomen, the skin being sodden, foul, and depilated ; some of the affected animals died in four to seven days ; others gradually recovered, the oedematous skin becoming hard, dry, and wrinkled. Some treated animals which survived the initial infection developed a large ulcer in the injured groin, with secondary infections of all kinds ; these ulcers often penetrated into the abdomen, eventually causing death between the seventh and sixteenth days. The appraisal of such deaths is difficult, but fortunately they were few in number. All animals were observed for thirty days ; but for the purpose of statistics we have taken an arbitrary period of ten days (which includes almost all the deaths), and only those occurring within that period have been shown in the protocols. In order to give a concise quantitative indication of the prolongation of life occurring among the non-survivors, an index figure (the guinea-pig-day index) has been calculated by adding up the number of guinea-pigs in the group which were alive on each of the first ten days after the initial operation and expressing this figure as the percentage of the maximum number of guinea-pig-days possible under this system—i.e., 60 in a group of 6 guinea-pigs. With *Cl. oedematiens*, however, it happened several times that the initial wound healed perfectly and the animal appeared quite

well until about the fifteenth day, when it died with the post-mortem appearances typical of this infection (i.e., massive gelatinous oedema of the subcutaneous tissues). Hence, with *oedematiens* the survival figures and the "guinea-pig-day index" are based on a period of twenty days.

The reopening of the wounds on the third day afforded an opportunity for inspection. In treated animals which would survive completely the wounds usually contained a trace of thin fluid ; pus was slight or absent. Parts of the damaged muscles were white and necrotic, but often the sloughs were surprisingly small in extent ; the rest of the muscles was vascular and bled easily. By this time sulphanilamide had all been absorbed, but sulphapyridine and sulphathiazole were found, in the appropriate animals, as small flattened masses. The action of the compounds is essentially bacteriostatic, not bactericidal. Smears made from the wounds on the third day invariably demonstrated great numbers of clostridia, which could readily be cultivated on appropriate media.

Preliminary experiments, made by inserting sulphanilamide, sulphapyridine, and sulphathiazole in solid form subcutaneously or intraperitoneally, showed that these compounds are comparatively harmless to the body tissues. By the naked eye no signs of local inflammation are seen ; microscopically a diffuse infiltration by polymorphs and monocytes is found, but this is not much more than would occur with any foreign body. Sodium sulphapyridine, however, is very irritant, being strongly alkaline. Similar conclusions concerning the innocuousness of sulphanilamide have been reached by Russell and Falconer (1940b), by Key and Burford (1940), and by Glynn (unpublished).

Prophylactic Action of Sulphonamide Compounds

In these experiments sulphanilamide, sulphapyridine, and sulphathiazole were used, and also a mixture (designated the "mixture" for brevity) of sulphanilamide 2 parts and sulphathiazole 1 part. It was hoped that this would combine the merits of the two components, sulphathiazole being the most potent and sulphanilamide being much cheaper and also more soluble and diffusible, which might be advantageous in the earliest stages of wound treatment. The power of the different compounds to prevent infection under these conditions is shown in Table I, in which the

TABLE I.—Protection of Guinea-pigs against Gas-gangrene Infections by Local Application of Sulphonamide Compounds. (The figures in brackets in each column represent the "guinea-pig-day" index.)

Strain	Survival of Animals				
	Sulphanilamide	Sulphapyridine	Sulphathiazole	Mixture	Controls
(a) <i>Cl. welchii</i> :					
Corcoran & Roshier	9/12 (86)	7/12 (80)	11/12 (93)	5/6 (88)	0/12 (3)
S.107 and A.7 ..	—	2/12 (21)	9/12 (77)	—	0/12 (1)
S.107 and S.R.12 ..	—	—	3/6 (50)	—	0/6 (0)
S.R.12 ..	—	3/6 (67)	—	—	0/6 (0)
S.R.12 ..	4/6 (73)	4/6 (82)	—	—	0/6 (3)
Hudson and Barnetson, plus contaminants*	3/12 (33)	1/12 (19)	5/12 (70)	8/12 (79)	0/12 (0)
Totals ..	16/30 (62) (53%)	17/48 (48) (35%)	28/42 (73) (67%)	13/18 (81) (72%)	0/54 (1) (0%)
(b) <i>Cl. septicum</i>					
V.S.20, V.S.A.48, and V.S.A.52 ..	0/23 (7) (0%)	9/24 (41) (38%)	18/24 (84) (75%)	7/17 (47) (41%)	0/24 (0.5) (0%)
(c) <i>Cl. oedematiens</i>					
No. 3 and Cossard	5/18 (41) (28%)	1/18 (10) (6%)	6/18 (38) (33%)	1/6 (21) (17%)	2/18 (14) (11%)

* In these two experiments the cultures of *Cl. welchii* were mixed with cultures of *B. proteus*, *B. coli*, *Ps. pyocyanea*, and *Cl. sporogenes* to imitate the conditions of a grossly contaminated wound.

results of different experiments are grouped together. In addition to a variation from one experiment to another, depending presumably on the virulence of the strain used, the number of bacilli introduced, the amount of toxin present, etc., the findings vary with the different organisms employed, so that it is necessary to consider each organism separately.

(a) *Cl. welchii*.—The culture usually contained over 10^8 bacilli per ml., and dilutions of 1/10,000 killed control animals when introduced into the wounds. Nevertheless the compounds usually saved a large proportion of the treated animals; and the life of non-survivors was often prolonged, as is shown by the "guinea-pig-day index." Since some of the experiments did not include all the three compounds, a simple inspection of the total survivals in each column does not provide an accurate picture of their relative potency; it is necessary to consider only those experiments in which each of the pair under comparison was present. Then, taking the three different pairs, consideration of the mixture being postponed, the following ratios of potency are obtained:

	Survivals	Average Guinea-pig-day Index
Sulphanilamide : sulphapyridine ..	16/30 : 12/30	60 : 56
Sulphapyridine : sulphathiazole ..	10/36 : 25/36	40 : 80
Sulphanilamide : sulphathiazole ..	12/24 : 16/24	60 : 80

Accordingly, sulphathiazole is distinctly more active against *Cl. welchii* than sulphapyridine, while sulphanilamide occupies an intermediate position. Another basis for comparison may be obtained by taking the totals of three experiments in which all four substances were tested and the infections in the wounds were especially virulent (not shown separately in table); the survivals were: controls 0/18, sulphapyridine 5/18, sulphanilamide 8/18, sulphathiazole 10/18, and "mixture" 13/18. The order of activity was the same as before; apparently the "mixture" was equal or superior to sulphathiazole, but with this number of animals only a tentative conclusion is possible.

(b) *Cl. septicæ*.—With this organism the cultures usually contained about 10^8 bacilli per ml. Sulphathiazole was again the most potent compound (survivals 75%); sulphapyridine was inferior (38%); sulphanilamide had practically no action against this organism (0%); while the "mixture" had an effect similar to that of sulphapyridine (41%).

(c) *Cl. oedematiens*.—The culture usually contained over 10^8 bacilli per ml.; dilutions of this amount by 1/100 often failed to kill control animals when introduced into the wounds. The survivals after sulphanilamide and sulphathiazole were somewhat more numerous than those among the controls, but not significantly so; and life was often prolonged, the differences between the "guinea-pig-day indices" being just great enough to be statistically significant (probability, 1:20). Probably sulphathiazole possesses a small prophylactic activity against infection with *Cl. oedematiens*, but it is much less than that against *Cl. welchii* and *Cl. septicæ*.

Controls.—In order to be sure that the survival of the treated animals was due to the specific action of the sulphonamide compounds and not to a non-specific action possessed by other powders a series of comparisons was made with various neutral compounds—namely, sodium chloride, calcium carbonate, and urea. As was expected, calcium carbonate or urea had no power at all to prevent infection, while sodium chloride appeared positively to favour its development.

Comparison with Other Anti-bacterial Compounds

Brief tests were also made on a number of other anti-bacterial compounds. So far as this short investigation went soluseptasine and prontosil soluble were inferior to sulphathiazole, possessing little or no activity. The survivals after proseptasine and prontosil rubrum were not much different in number from those after sulphathiazole; but the clinical condition of the wounds (many of which showed a limited spread of the gangrenous infection) was definitely worse. None of these four compounds seemed promising enough to warrant more extended investigation. A number of animals were treated with nitrobenzene-sulphonamide; the results indicated that this compound is highly toxic, and that *in vivo*, under these experimental conditions, its activity is no greater than that of sulphathiazole. Experiments were also made with proflavine on account of the recommendations which this compound has

received as a non-irritant antiseptic for wounds in recent papers—e.g., Russell and Falconer (1940a). Applied in strong concentration proflavine will certainly prevent infection of a wound by anaerobes subsequently inserted. On reopening at the third day, wounds which had been treated with 1% or 0.1% solutions showed fairly healthy tissues and only small numbers of bacilli. Nevertheless, in view of the mediocre results obtained with acridine compounds in the last war (presumably through inability to reach bacteria situated inside the tissues), the usefulness of proflavine as a wound antiseptic is probably limited.

Comparison of Local and Systemic Treatment

Guinea-pigs treated locally were compared with those which received sulphathiazole intraperitoneally, given as a suspension in water, about two hours before the wound was made. The dose was 0.2 gramme per guinea-pig of 200 to 250 grammes. This was about the maximum tolerated dose for infected animals: healthy animals would stand 0.4 or sometimes 0.8 gramme intraperitoneally, but infected ones were apt to show toxic symptoms after 0.4 gramme. In any case much of the drug was not absorbed during the first two days, but was deposited as small masses throughout the abdomen. (When sulphonamides are inserted into a wound, however, the toxicity depends more upon the rate of absorption than upon the total quantity injected; under these circumstances guinea-pigs will tolerate over 1 gramme of sulphathiazole.) The results of this series are given in Table II. The difference

TABLE II.—Comparison of Activity of Sulphathiazole in Protecting Guinea-pigs against Gas-gangrene Infections when Applied Locally and when Administered Systemically by Intraperitoneal Injection. (The figures in brackets in each column represent the "guinea-pig-day index.")

Infection	Survival of Animals		
	Local Treatment	Intra- peritoneal Treatment	Controls
<i>Cl. welchii</i> (4 strains) ..	14/24 (74)	5/14 (46)	0/24 (0)
<i>Cl. septicæ</i> (2 strains) ..	7/12 (67)	0/8 (3)	0/12 (0)
Totals	21/36 (71) (58%)	5/22 (30) (23%)	0/36 (0) (0%)

in efficacy between the two treatments is greater with *Cl. septicæ* than with *Cl. welchii*; but, taking the two together, it is seen that while local treatment saved 58% of the animals, systemic (intraperitoneal) treatment, even if given two hours previously, saved only 23%. Thus, although systemic treatment has some value it is inferior to local treatment under these experimental conditions.

Effect of Delaying Local Treatment

The effect of delaying local treatment was studied in four experiments with *Cl. welchii* and one with *Cl. septicæ*. In these animals the wound was made in the usual manner and the cloth soaked in culture inserted. After two hours or six hours the incision was reopened, the cloth lifted out, the wound packed with 0.2 gramme of sulphathiazole, and the cloth replaced. Taking the series as a whole it was found that when treatment was immediate 20 out of 30 animals survived (67%); when the treatment was delayed two hours 3 out of 15 animals survived (26%); and when treatment was delayed six hours none out of 6 animals survived. None of the 30 controls survived. Thus a delay of even two hours greatly reduced the efficacy of the treatment.

Diffusion of Sulphonamide Compounds from Wounds

The time required for absorption of the compounds from wounds and the concentrations which occurred in adjacent tissues were studied in two ways. First, pre-

liminary experiments were made to see how far the high concentration present in the wound cavity extended through the adjacent tissues. A transverse incision was made into the lumbar muscles of rabbits (weight about 1 kg.), and 0.25 gramme of sulphanilamide, made into a thick paste with water, was inserted. The fascial covering of the muscle was carefully sutured together and any extruded sulphanilamide wiped away. The skin wound was then closed. Six or twenty-four hours later the rabbits were killed, and transverse slices of the lumbar muscles about 0.5 cm. thick were taken at various distances from the wound; blood from the heart and pieces of muscle from the opposite thigh were also taken as controls. The concentration of free sulphanilamide in the various specimens was estimated by the method of Marshall and Litchfield (1938). The results of an experiment comprising six rabbits are given in Table III. At six hours

TABLE III.—*Diffusion of Sulphanilamide from an Uninfected Wound in Rabbit's Muscle*

Time	Concentration of Free Sulphanilamide (mg. per 100 c.cm.)				
	Sides of Wound	1-2 cm. Away	4-5 cm. Away	Control Muscle (Opp. Thigh)	Blood
6 hours	105+	16.2	2.6	2.2	0.7
	122+	4.9	5.0	3.8	1.3
	109+	7.0	3.2	5.1	1.8
Average	112+	9.4	3.6	3.7	1.3
24 hours	0.12	0.2	0.13	0.1	0
	0.3	0.1	0.13	0.13	0
	0.3	0.13	0.6	0.1	0
Average	0.24	0.14	0.29	0.11	0

solid sulphanilamide was still present in the wound, but after twenty-four hours practically all the sulphanilamide had disappeared. At six hours the concentration in muscle 4 to 5 cm. away from the wound was no greater than that in muscle elsewhere; muscle 1 to 2 cm. away from the wound appeared to have a higher concentration, but part of this may have been due to leakage from the wound between the surface of the muscle and its fascial covering. At any rate, the higher concentration did not extend to any great distance from the wound. The fact that the concentration of free sulphanilamide in the control muscles of these animals was higher than that in the blood was unexpected, but similar findings were obtained in confirmatory experiments.

The second series of experiments was made to investigate the fate of sulphonamide compounds in the experimental wounds of guinea-pigs described above. The wounds were formed in the standard manner, and 0.2 gramme of the various compounds was inserted; they were then closed without inserting the piece of cloth. The animals were killed at suitable intervals, the reopening of the wound on the third day being omitted. The muscles of the wounded thigh, the muscles of the opposite thigh (control), and blood from the heart were taken for chemical estimation. The results of these experiments are given in Table IV, and the average concentration of the various compounds in the wound is depicted graphically in the figure. Sulphanilamide is rapidly absorbed, so that its influence in the wound is probably slight after the first twenty-four hours. Solid sulphapyridine persists in the wound for seven to ten days; its solubility in 0.85% saline at 37° C. is 42 mg. per 100 ml. Solid sulphathiazole persists in the wound over four days; during this period its concentration in the wound fluids will be higher than that of sulphapyridine, owing to its greater solubility—namely, 104 mg. per 100 ml. in 0.85% saline at 37° C. (Ewins, personal communication). This greater solubility may be a partial explanation of its greater therapeutic potency (described above), but part also is due to its

more powerful bacteriostatic action against anaerobes, which can be demonstrated *in vitro* (Fuller, personal communication). By a suitable mixture of the more diffusible sulphonamides—e.g., sulphanilamide and sulphathiazole—

TABLE IV.—*Concentration of Sulphonamide Compounds in Guinea-pigs (Weight 200 to 250 grammes), following Insertion of 0.2 gramme of Drug into Experimental Wounds*

Day	Concentration (mg. per 100 ml.)								
	Sulphanilamide			Sulphapyridine			Sulphathiazole		
	Wound	Control Muscle	Blood	Wound	Control Muscle	Blood	Wound	Control Muscle	Blood
1	3.0	0.37	0.21	S (42)	2.1	2.4	S (104)	2.4	1.2
2	3.0	0.21	0.07	S (42)	1.5	1.5	S (104)	0.38	0.6
3	1.8	0.33	0	S (42)	0.8	0.3	S (104)	1.1	0.5
4	—	—	—	S (42)	3.2	1.3	S (104)	0.7	0.7
7	—	—	—	S (42)	0.5	0.5	—	0.08	0.03
10	—	—	—	27.6	0.2	—	8.3	0.5	—
11	3.7	0.2	—	9.9	0.3	—	2.1	0.06	—
18	1.2	0.04	—	3.1	0.02	—	1.7	0.1	—
28	—	—	—	0.4	0.3	—	0.5	0.3	—

The figures given are the average for groups of three animals. S indicates that solid compound was present.

Graph showing concentrations of sulphonamides in wounds of guinea-pigs.

with the less diffusible ones—e.g., sulphapyridine and prosectasine—it would be possible to produce a combination which would embody the advantages of both types—namely, the high initial concentration of the former and the prolonged action of the latter. However, so far as infection of wounds with *Cl. welchii* or *Cl. septique* is concerned, it is probably the initial period which is the important one: if the organisms can be held in check for the first two to four days it is less likely that gas gangrene will develop.

Comparison of Drug Treatment with Treatment by Antisera

A number of the animals were also treated by antisera. The wound was made according to the standard technique, the antiserum being given by intraperitoneal injection shortly before or after the operation; on the third day the wound was reopened to remove the cloth, and the dose of antiserum was repeated. It is impossible to reproduce human conditions exactly, since among other difficulties there is no agreement as to what is an equivalent dosage of a serum for a guinea-pig and for a man. Two series of experiments were made. In the first (Table V) use was made of three concentrated monovalent sera kindly supplied by Burroughs Wellcome and Co.: (1) prepared against *Cl. welchii*, S.R.12 (Type A)—4,000 units of antitoxin per ml.; (2) prepared against *Cl. septique*, V.S.14—6,000 units of antitoxin per ml.; (3) prepared against *Cl. oedematiens*, No. 3—9,000 units of antitoxin per ml. The doses given are probably much larger than is necessary. With *Cl. welchii* the antiserum gave complete protection in one case and no protection in the other; it is not clear whether this was due to the different strain used or to the delay in administering treatment. With *Cl. oedematiens* all the animals treated by serum survived except for 2 which died seventeen days after the second dose:

TABLE V.—Comparison of Activity of Sulphathiazole in Protecting Guinea-pigs against Gas-gangrene Infections with that of Specific Monovalent Sera (Antitoxin)

Infection		Antitoxin		Survival of Animals		
Strain	Bacilli per ml. $\times 10^8$	Dose Units	Time	Anti-toxin	Sulpha-thiazole	Controls
<i>Cl. welchii</i> , S.107, incompletely washed	23	1,000 i.p.	$\frac{1}{2}$ hr. before	3/3 (100)	5/6 (83)	0/6 (2)
<i>Cl. welchii</i> , A.7, incompletely washed		1,000 i.p.	2 $\frac{1}{2}$ hrs. after	0/3 (0)	4/6 (72)	0/6 (0)
<i>Cl. septique</i> , V.S.14, washed	<0.5	500 i.v. 1,500 locally	Just before Just before	3/3 (100) 3/3 (100)	3/3 (100) (sulpha-pyridine)	1/3 (40)
<i>Cl. oedematiens</i> , No. 3, washed	<0.5	9,000 i.p.	1 $\frac{1}{2}$ hrs. before	5/6 (99)	2/6 (43)	1/6 (20)
<i>Cl. oedematiens</i> , No. 3, incompletely washed	1.8	1,000 i.p.	$\frac{1}{2}$ hr. after	2/3 (99)	4/6 (67)	1/6 (18)

presumably, if treatment had been continued at five-day intervals these deaths would not have occurred.

The second series of experiments was designed to imitate more closely the conditions in a casualty department when a small dose of antitoxin is given prophylactically against an infection of unknown identity. A commercial polyvalent serum was taken containing *Cl. welchii* antitoxin 3,000 units, *Cl. septique* antitoxin 1,500 units, and *Cl. oedematiens* antitoxin 1,000 units per ml. This was injected intraperitoneally at the time of the operation or at various subsequent periods. Many different strains of clostridium were used to infect the wound; in some experiments two different strains were inserted simultaneously (Table VI). The most striking features are the

TABLE VI.—The Activity of a Polyvalent Serum (Antitoxin) in Protecting Guinea-pigs against Gas-gangrene Infections

Infection		Dose of Serum (ml.)	Survival of Animals					Controls
Strain	Bacilli per ml. $\times 10^8$		Antitoxin				Sulpha-thiazole	
			Before	After 1 hr.	After 2 hrs.	After 6 hrs.		
<i>Cl. welchii</i> A.5	>10	0.3	0/2 (20)					0/2 (0)
S.R.12	3	0.3	2/2 (100)		3/3 (100)	3/3 (100)	3/3 (100)	0/3 (0)
S.107	>10	0.3	1/3 (50)				0/3 (0)	0/3 (0)
Rosher	8	0.3	2/2 (100)					0/2
Hudson & Barnetson	>10	0.3	3/3 (100)		0/3 (23)	0/3 (0)		0/3 (0)
Constantine	7	0.3	2/2 (100)					0/2 (0)
<i>Cl. septique</i> V.S.14 and V.S.20	3	0.3	0/2 (0)					0/2 (0)
V.S.A.48	1	0.6	0/2 (0)	0/2 (0)	0/2 (0)		6/6 (100)	0/6 (0)
V.S.A.48 and V.S.A.52	1.5	0.3			0/3 (0)			0/3 (0)
<i>Cl. oedematiens</i> No. 3	2	0.2	1/2 (67)					0/2 (5)
Cossard	5	0.3	0/2 (50)					0/2 (5)

comparative success of this antiserum in protecting the animals against *Cl. oedematiens* (against which drugs are ineffective) and its failure against *Cl. septique* (for which sulphathiazole is potent). Against *Cl. welchii* the results varied according to the different strains. Delay in the treatment in one case (Hudson and Barnetson) annulled its efficacy. Since the serum was administered intraperitoneally in all these animals there may have been some delay before a sufficient amount was absorbed into the blood stream and carried to the wound. A more detailed discussion of these results with serum is beyond the scope of this paper.

Discussion

The information obtained experimentally about the prevention of gas gangrene in wounds by the local

application of sulphonamide compounds may be briefly summarized thus: Sulphonamides will protect against *Cl. welchii* and *Cl. septique*, but only to a slight extent against *Cl. oedematiens*; the most potent compound under these experimental conditions is sulphathiazole; treatment by local application is more effective than systemic treatment; delay in applying the treatment reduces its value considerably. These results concerning the superior potency of sulphathiazole and the difficulty of combating infections with *Cl. oedematiens* agree with the experience obtained in mice by McIntosh (above) and by Stephenson (personal communication). The findings are more favourable than those originally reported by Legroux, although his infections appear to have been less severe, since his controls lived for two to three days while those in the present work died in twenty-four hours.

The fundamental purpose of all this research was to obtain information as to how far the insertion of sulphonamide compounds into wounds would be a useful addition to the surgical methods now employed. Such insertion could occur under three different conditions: (1) On the battlefield, with little disturbance of the wound, for the purpose of delaying the development of infection in cases which will not reach a surgeon for a long period or in which surgical treatment must be postponed on account of shock (Buxton, 1940). (2) In the operating theatre, after débridement, etc. (possibly followed by plaster-of-Paris), for the purpose of inhibiting the development of organisms (anaerobic or pyogenic) which cannot be excised. (3) At subsequent dressings of wounds (this will probably be directed mainly against streptococci).

The experiments were designed to resemble the first two of these groups; but admittedly the conditions are somewhat artificial, and a simple transference of the conclusions to human cases is not quite legitimate. However, they do suggest strongly that treatment along these lines would be beneficial to casualties. Such treatment can be recommended the more confidently since the compounds are innocuous and non-irritant. A number of air-raid casualties and street accidents have been treated in this way at adjacent hospitals, and the results have been extremely satisfactory, even in wounds with a heavy initial contamination.

So far as gas gangrene is concerned, the treatment may be expected to be of value chiefly as a prophylactic measure, and much less so as a therapeutic one; and clearly it should be a supplement to proper surgical measures and not a substitute for them (unless they are not available). In dealing, however, with a condition such as gas gangrene, which although often fatal has a low and irregular incidence, it will be very difficult to get reliable evidence about the value of such prophylactic treatment in human cases. At a recent conference the incidence of gas gangrene among air-raid casualties was given as 5 in 1,000 (0.5%) in one town and 5 in 187 (2.7%) in a hospital in another. Assuming an incidence of 1.5%, and that a prophylactic treatment applied to all patients caused a diminution by 80%—that is, an incidence rate of 0.3%—then to get the barest significant result (probability of chance, 1:22), and using the strictest precautions to obtain identical patients in both series, two sets of cases each about 320 in number would be required. Under actual clinical conditions, even if great care were exercised, a much larger number would be needed. Consequently an assessment of the value of this treatment by purely clinical methods will take a long time, even with the abundance of material which war unfortunately provides.

Finally, a word on the prophylaxis of gas gangrene by means of serum—although this question lies mainly outside the scope of the present paper. Since serum and

drug supplement one another, the ideal procedure for general use should include oth. In special hospitals, however, where large numbers of casualties are seen, it would be desirable to build up three series of cases—treated respectively with drug, with serum, and with neither (controls)—so that the clinical assessment on these procedures (discussed above) could eventually be made.

Summary

Experimental wounds (with crushed muscle and the presence of a foreign body) were made in guinea-pigs, and large numbers of gas-gangrene organisms were inserted; untreated animals died within twenty-four hours.

Application of sulphonamide compounds to the wounds at the same time as insertion of the organisms saved a large proportion of the animals infected with *Cl. welchii* and with *Cl. septicus*, but it had only slight influence upon infections with *Cl. oedematiens*. Sulphathiazole was the most effective compound to use in all cases; sulphanilamide was superior to sulphapyridine in preventing infection with *Cl. welchii*, but it had practically no activity against *Cl. septicus*.

Local application of sulphathiazole to the wound was more effective in preventing infection than systemic administration by intraperitoneal injection. Delay of two hours in applying the treatment greatly reduced its effectiveness.

Sulphanilamide is absorbed from wounds very quickly (in less than twenty-four hours), giving a high but transitory concentration in the fluids of the wound (1,500 mg. per 100 c.cm.); sulphapyridine is absorbed slowly from the wound (seven to ten days) and its concentration in the wound is low (42 mg.) but prolonged; sulphathiazole occupies an intermediate position, being absorbed in about five to six days and producing a concentration of about 104 mg.

The development of the infection in these wounds could also be prevented by administration of sera (antitoxin). Monovalent sera specific for *Cl. welchii*, *Cl. septicus*, or *Cl. oedematiens* protected the guinea-pigs against these organisms respectively. A commercial polyvalent serum gave protection against *Cl. welchii* (most strains) and *Cl. oedematiens*, but not against *Cl. septicus*.

It is recommended that war wounds should be protected against the development of gas gangrene by the local application of sulphonamide compounds together with injection of antitoxin.

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ACTIVATION OF THROMBOPLASTIN PREPARATIONS BY HAEMOLYSIS AND BY LECITHIN

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During an investigation into the effect of storage on the prothrombin index of blood (Crosbie, Scarborough, and Thompson, 1941) one of us (A. C.) observed that the presence of haemolysis in the plasma was often associated with a decreased coagulation time as determined by Quick's technique. This acceleration was more pronounced when Russell-viper venom was used instead of brain extract as the source of thromboplastin as recommended by Fullerton (1940). While seeking an explanation for this phenomenon we read the letter of Hobson and Witts (1940) stating that they had confirmed the original observation of Trevan and Macfarlane (1936-7) that the coagulant action of Russell-viper venom was potentiated by the addition of lecithin. It occurred to us that the accelerating effect of haemolysis might be due to the liberation of lecithin into the plasma from the ruptured envelopes of erythrocytes. In this paper, a summary of which has already appeared (Crosbie and Scarborough, 1941), we give an account of our results. While the paper was being prepared for the press Witts and Hobson (1940) published their own observations, which demonstrated that lecithin used in association with Russell-viper venom produces an acceleration of the coagulation time as determined by Quick's method, and suggest that the adjuvant action of lecithin may be used in the elucidation of three types of haemorrhagic tendency.

Material and Methods

None of the subjects from whom blood was taken for this investigation had any form of haemorrhagic disease. Apart from a few patients with jaundice, to whom specific reference is made, the subjects either attended the out-patient department for minor complaints or were normal healthy adults. All coagulation times were determined by the method of Quick (1936), except that snake venom or venom plus lecithin replaced brain extract as the source of thromboplastin.

4.5 c.cm. of venous blood withdrawn with the minimum of trauma was added to 0.5 c.cm. of M/10 potassium oxalate or, in some cases, to 0.5 c.cm. of 3.8% sodium citrate. In order to reduce cell damage to the minimum, centrifugalization was avoided and plasma was obtained by allowing the specimens to stand overnight in a refrigerator at 2° to 5° C. This treatment does not affect the coagulation time as determined by Quick's original method with brain extract as the source of thromboplastin. 0.1 c.cm. of plasma was pipetted into a clean dry test-tube (7 × 70 mm.) in a water bath at 37° C. and mixed with 0.1 c.cm. of thromboplastin preparation. Immediately 0.1 c.cm. of M/40 calcium chloride was added and the coagulation time at 37° C. was determined in seconds.

Three sources of thromboplastin were used:

1. Brain Extract.—This was prepared according to the method of Quick, but from human instead of from rabbit brain as originally recommended.
2. Russell-viper Venom (stypven, B. W. and Co.).—Two different batches of material were used. They did not differ appreciably in activity. A dilution of 1 in 10,000