THE BACTERIOSTATIC EFFECT OF DISINFECTANTS IN HUMAN SERUM AND CITRATED PLASMA

BY

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Although human serum is not a good medium for bacterial growth, bacteria will remain viable in it, and certain organisms will grow in it even at temperatures as low as 0° C. This is also true for reconstituted dried serum, so that bacterial infection is a potential danger in the use of reconstituted dried serum as a transfusion fluid—particularly since during the process of solution serum may become contaminated either through careless handling or by the use of non-sterile water in dissolving the serum. It is improbable that serum contaminated in this way would be a danger to the patient if it were used immediately after reconstitution, but if it were allowed to stand under bad conditions of temperature bacterial growth would take place. Therefore an attempt has been made to find a disinfectant which could be added to the serum before drying, or to the dissolving fluid, in a concentration that would be bacteriostatic and yet within the limits of toxicity even when large volumes of serum were transfused. At a later stage an experiment on citrated plasma was included.

During the course of the experiments it was found that *Staph. aureus*, *Micrococcus tetragenus*, and an enterococcus grew readily in serum from an original inoculum of 20 organisms per c.cm. Under similar conditions no growth of anthrax, streptococci of the "Oakley" strain, *Bact. aertrycke*, *Bact. shigae*, or *Bact. coli* could be demonstrated, while *B. subtilis* grew in serum after a preliminary delay during which the presence of living organisms could not be proved. The work was therefore carried out using *Staph. aureus*, *M. tetragenus*, *B. subtilis*, and an enterococcus as contaminating organisms in the serum. These organisms might reasonably be expected to be found as contaminants in stored serum or plasma, and members of the *subtilis-megatherium* group, micrococci, and pseudomonas have been isolated from serum received at this laboratory for drying.

A disinfectant to be used for the preservation of stored serum should be soluble enough to be added in a concentration that will not dilute the serum unduly. It must not react with serum proteins to alter their physical characteristics, and must be active as a bacteriostatic within the limits of toxicity of the drug even when large volumes of fluid are to be used. During the course of the experiments eight disinfectants were tried. These included four mercurials—merthiolate, metaphen, phenyl mercuric nitrate, and phenyl mercuric acetate; also proflavine, sulphanilamide, thymol, and *p*-chlor-*m*-cresol.

Effective Range of the Disinfectants

The effective range of concentration of each disinfectant was first determined. To 0.9 c.cm. of serum or reconstituted dried serum was added 0.1 c.cm. of a suitable concentration of disinfectant, depending on its solubility in water; from this mixture a series of falling dilutions was made in serum. In the case of metaphen four doubled dilutions were made, but with the other disinfectants four or five decimal dilutions were used, and of each dilution three tubes were set up. To each tube 0.02 c.cm. of a saline suspension containing 2,000, 200, or 20 organisms was added, and a control tube containing 1 c.cm. of serum without disinfectant was inoculated with each suspension. The tubes were incubated at 37° C., and growth was tested by making a stroke inoculation on an agar

plate at intervals. Saline suspensions of bacteria were prepared by washing the growth from a twenty-four-hour agar slope culture and diluting with sterile saline until the suspension contained 1,000 million organisms per c.cm. measured by opacity. Dilutions of 1/1,000, 1/10,000, and 1/100,000 were made from this, and one drop from a fifty-drop pipette was used as an inoculum so that each drop contained 2,000, 200, or 20 organisms. The test organisms used in this series were *Staph. aureus* and *B. subtilis*.

Results of a typical experiment are shown in Table I, which indicates that 1/125,000 is the minimal concentration of phenyl

TABLE I.—Phenyl Mercuric Nitrate: Three Days' Incubation at 37° C.

Growth Medium	Organism + Inoculum	Dilution of Disinfectant				Control (no Disinfectant)
		1/12,500	1/125,000	1/1,250,000	1/12,500,000	
Filtered serum	<i>Staph. aureus</i> 20/c.cm.	—	—	—	+	+
	200/c.cm.	—	—	+	+	+
	2,000/c.cm.	—	—	+	+	+
Reconstituted dried serum	20/c.cm.	—	—	+	+	+
	200/c.cm.	—	—	+	+	+
	2,000 c.cm.	—	—	+	+	+

+, growth on subculture; —, no growth.

mercuric nitrate capable of preventing the growth of *Staph. aureus* in serum. The results for tests on the eight disinfectants are presented in Table II; the effect of size of

TABLE II

Disinfectant	Range of Dilutions Tested	Medium	Test Organism	Incubation Time (days)	Highest Dilution to Inhibit Growth
Metaphen ..	1/20,000 to 1/160,000	Reconstituted dried serum	<i>Staph. aureus</i>	1	1/80,000
				4	1/80,000
				6	1/80,000
		Filtered serum	<i>B. subtilis</i>	1	1/160,000
				4	1/160,000
				6	1/160,000
Merthiolate ..	1/10,000 to 1/10,000,000	Filtered serum	<i>Staph. aureus</i>	1	1/100,000
				6	1/100,000
				1	1/1,000,000
		Reconstituted dried serum	<i>B. subtilis</i>	1	1/100,000
				6	1/100,000
				1	1/100,000
Phenyl mercuric nitrate ..	1/12,500 to 1/12,500,000	Filtered serum	<i>Staph. aureus</i>	1	1/125,000
				6	1/125,000
				1	1/1,250,000
		Reconstituted dried serum	<i>B. subtilis</i>	1	1/125,000
				6	1/125,000
				1	1/1,250,000
Phenyl mercuric acetate ..	1/10,000 to 1/1,000,000	Filtered serum	<i>Staph. aureus</i>	1	1/1,000,000
				3	1/1,000,000
				1	1/1,000,000
		Reconstituted dried serum	<i>B. subtilis</i>	1	1/1,000,000
				3	1/1,000,000
				1	1/100,000
Proflavine ..	1/1,000 to 1/1,000,000	Filtered serum	<i>Staph. aureus</i>	1	1/100,000
				4	1/100,000
Thymol ..	1/2,000 to 1/2,000,000	Filtered serum	<i>Staph. aureus</i>	1	Growth in 1/2,000
			<i>B. subtilis</i>	1	Growth in 1/2,000
<i>p</i> -chlor- <i>m</i> -cresol	1/2,000 to 1/2,000,000	Filtered serum	<i>Staph. aureus</i>	1	1/200,000
				3	1/2,000
				1	Growth in 1/2,000

inoculum has been neglected, since no gross differences could be demonstrated by varying the size of inoculum between 20 organisms and 2,000 organisms per c.cm. As is shown in Table II, the lowest concentration of disinfectant in serum which will inhibit growth has been determined.

Testing for Viable Organisms

In a second series of experiments an attempt has been made to see whether viable organisms were still present despite the absence of growth in the serum. So viable counts were

carried out on reconstituted dried serum inoculated with a suitable organism and incubated at 37° C. with the highest dilution of disinfectant which in the experiments described in the previous paragraph had been shown to inhibit bacterial growth. In this series the inoculum was always 20 organisms per c.cm.

Method.—To 9 c.cm. of reconstituted dried serum made up to normal concentration 1 c.cm. of a suitable dilution of disinfectant was added so that the final disinfectant-serum mixture contained a dilution of disinfectant which had been shown to inhibit growth (Table II). Each tube was inoculated with 0.2 c.cm. of a saline suspension of bacteria, prepared as before, the final concentration of bacteria being 20 organisms per c.cm. Control tubes containing 10 c.cm. of serum without disinfectant were inoculated with 0.2 c.cm. of the same suspension; the tubes were incubated at 37° C. Similar tubes were set up using citrated plasma as the growth medium for the bacteria. Viable counts were carried out by Miles's (Aitken, Barling, and Miles, 1936) "surface viable" technique after variable periods of time, but if growth in the serum-disinfectant mixture could be demonstrated at an early stage further counts were omitted. Three decimal dilutions were made in sterile saline, and a drop from a 50-drop pipette was allowed to fall on each of three marked places on an over-dried agar plate; each count is the average of three such drop areas containing a suitable number of colonies. Similar counts were carried out on control sera. As a routine the sera were inoculated with *Staph. aureus*, *B. subtilis*, and an enterococcus. Drop areas of *Staph. aureus* and enterococcus containing up to 60 colonies could be counted accurately, but the *subtilis* colonies were very large, and from 8 to 20 only could be counted. The results with this organism are therefore less accurate. The method of testing for viable organisms by plating from a ten-times dilution of the serum-disinfectant mixture was based on the assumption that by diluting the serum-disinfectant mixture ten times the concentration of disinfectant becomes low enough to allow inhibited

but still viable organisms in the original disinfectant mixture to grow in the lower concentration. Thus serum containing merthiolate 1/100,000 inhibited the growth of *Staph. aureus*, but, on diluting, these organisms were able to grow. The dilution to 1/1,000 was made in order to get drop areas which would contain a countable number of colonies.

Results.—A summary of results is presented in Table III. In the case of merthiolate and phenyl mercuric nitrate (1/100,000) growth of bacteria could be demonstrated on diluting the serum-disinfectant mixture, so that fresh experiments were set up. Merthiolate was tried in a dilution of 1/10,000 because that dilution was already commonly used in the preservation of citrated plasma and was found to be effective in this concentration. Phenyl mercuric nitrate, except when the serum was contaminated with enterococcus, was effective in a concentration of 1/50,000.

Effects of Use of Disinfectants

Effect of Drying Disinfectant with Serum.—Sulphanilamide, metaphen, phenyl mercuric nitrate, and proflavine were added to the serum before drying, and the bacteriostatic power was estimated after reconstitution. So far as could be seen, freezing at -20° C. and drying had no effect either on the bacteriostatic power of the disinfectant or on the solubility of the serum. Bacterial counts were also made on serum which had been made up to four times the normal concentration on reconstituting. Again no difference in bacteriostatic power of the disinfectant could be detected.

Effect on *Pseudomonas pyocyanea*.—In a bottle of reconstituted dried serum containing phenyl mercuric nitrate 1/10,000, from which a sample of serum had been taken with a chemically clean pipette for an osmotic pressure estimation, *Ps. pyocyanea* was found to be growing profusely. A loopful of this serum was added to serum containing 1/10,000 proflavine, and a loopful of a saline suspension of this organism was added to dried serum containing merthiolate, metaphen.

TABLE III.—Growth in the Presence of Disinfectant

Organism	Medium	Incubation Time (Days)	Control (no Disinfectant)	Sulphanilamide 1/5,000	Phenyl Mercuric Nitrate	Phenyl Mercuric Acetate	Metaphen	Merthiolate	Proflavine
M. tetragenus ..	Dried serum reconstituted to 4 × normal	1 2 7	47,000,000 466,000,000 545,000,000	No growth 4,150 53,000,000					
Staph. aureus ..	"	1 2 7	201,500 24,600,000 91,500,000	No growth 9,000 2,900,000					
B. subtilis ..	"	1 2	5,000 2,500,000	No growth					
Enterococcus ..	Dried serum, normal concentration	1	210,000,000	1,800,000					
Staph. aureus ..	Dried serum, normal concentration	1 3 7	220,000 15,500,000 18,500,000				1/10,000 No growth " " "		
B. subtilis ..	"	1 3 7	No growth 1/100 450,000 60,000,000				" " "		
Enterococcus ..	"	1	805,000,000				140,000		
Staph. aureus ..	Dried serum, normal concentration	3 9	200,000 13,000,000		1/100,000 No growth Growth + 200,000	1/100,000 2,000,000 No growth		1/100,000 17,800 2,000 Uncountable 1/1,000	1/100,000 No growth 512,000 No growth " Uncountable 1/1,000
B. subtilis ..	"	3 9	130,000 600,000						
Enterococcus ..	"	3 9	58,000,000 10,000,000		700,000	" "			
Staph. aureus ..	Dried serum, normal concentration	2 7	1,800,000 10,500,000		1/50,000 No growth "			1/10,000 No growth "	
B. subtilis ..	"	2 7	28,000 175,000		" "			" "	
Enterococcus ..	"	2 7	210,000,000 20,000,000		115,000			" "	
Staph. aureus ..	Citrated plasma	3 8	10,500 8,300,000	No growth 10,000,000 (?)	1/50,000 No growth "		1/10,000 No growth "	No growth "	No growth "
B. subtilis ..	"	3 8	No growth Profuse growth	No growth "	" "		" "	" "	" "
Enterococcus ..	"	3 8	1,900,000,000 48,000,000	Uncountable 1/1,000	Uncountable 1/1,000		Uncountable 1/1,000	" "	" "

and phenyl mercuric acetate, so that the final concentration was 1/10,000. Growth of the organism could be demonstrated in three days at room temperature. Apparently none of these disinfectants will control the growth of pseudomonas.

Effect on Moulds.—The inhibitory effect of proflavine and phenyl mercuric nitrate on moulds was tried in one experiment. A series of half dilutions of disinfectants was made in reconstituted dried serum from 1/10,000 to 1/160,000, and these were inoculated with a small amount of the vegetative form of an air-borne mould growing in serum broth. At room temperature growth took place in all dilutions of the serum containing proflavine, but was inhibited by 1/80,000 phenyl mercuric nitrate. There was no apparent difference in the tubes containing proflavine and in the controls.

Effect of Sulphanilamide in Serum and Blood.—Because of our inability to repeat the results of Novak (1939) one experiment was carried out on the lines suggested by Novak and Hunwicke (1940). Into twelve tubes 40 c.cm. of sterile citrated human blood was measured. Four were inoculated with a saline suspension of *B. subtilis*, four with *M. tetragenus*, and four more with *Ps. pyocyanea*. The suspensions were so adjusted that the blood contained 20 organisms per c.cm. Two tubes of each group were kept as a control, and to the others sulphanilamide was added, the final concentration being 1/5,000. One of each of the tubes containing organism and sulphanilamide, and its appropriate control, were held at room temperature, and the second set at 4° C. The blood was tested for bacterial growth after four days and seventeen days by smearing a loopful on the surface of an agar plate. After thirty days' incubation poured plates were made, those of the blood held at 4° C. with 1 c.cm., those held at room temperature with 0.1 c.cm. After four days at room temperature evidence of growth was obtained in the control tube containing *B. subtilis*, and after seventeen days growth in the tube containing *M. tetragenus* and *Ps. pyocyanea* was demonstrated. The plates poured after thirty days showed profuse growth of the bacteria in all tubes, and there was no obvious difference between those which contained sulphanilamide and the controls. With the exception of the control tube containing *Ps. pyocyanea* there was no growth in any of the tubes held at 4° C. After fifty-four days these tubes were incubated at 37° C. for three days and plates poured, using 1 c.cm. of blood as an inoculum. Profuse growth was observed in the plates from the control tubes, but there was no growth in any tube which contained sulphanilamide. Except that no growth of the controls could be shown at 4° C., these results corroborate those reported by Novak and Hunwicke.

Conclusions

As had been expected from Fildes's (1940) work the bacteriostatic power of mercurial disinfectants was considerably less in serum than in other media. Birkhaug (1930) has reported that metaphen in serum broth will destroy *Staph. aureus* in a dilution of 1/120,000. In serum a dilution of 1/80,000 allowed definite bacterial growth, and 1/40,000 proved only bacteriostatic—that is, bacteria would grow when the serum was diluted.

The efficacy of the individual disinfectants tried in these experiments appeared to depend entirely on the contaminating organism. *Metaphen* in a dilution of 1/10,000 inhibited the growth for six days of *Staph. aureus* and *B. subtilis* in serum, but enterococcus and *Ps. pyocyanea* grew in this concentration. *Metaphen* is soluble in dilute alkali (pH 9) in a concentration of 1/500, and does not affect the physical nature of the serum (Birkhaug, 1934; Hirschfelder and Wright, 1930). Bernstine (1929) has reported the intravenous injection of 10 mg. of metaphen without toxic effects. It will be observed, however, that the transfusion of 1 litre of serum would involve the injection of 100 mg. of the disinfectant.

Merthiolate had an activity of the same order as metaphen, except that no growth of enterococcus could be demonstrated in six days with a dilution of 1/10,000. This dilution did not prevent the growth of *Ps. pyocyanea*. *Merthiolate* is relatively non-toxic, and does not affect serum proteins (Powell and Jamieson, 1931).

Phenyl mercuric nitrate was effective against *Staph. aureus* and *B. subtilis* in a dilution of 1/50,000, but this dilution did not prevent the growth of enterococcus after six days, and

1/10,000 had no effect on the growth of *Ps. pyocyanea*. The growth of mould was inhibited by 1/80,000. The pH of the medium did not affect the bacteriostatic powers of this disinfectant. Drying the serum in the presence of 1/10,000 phenyl mercuric nitrate had no effect on the osmotic pressure after reconstitution (Adair, personal communication). Phenyl mercuric nitrate is reported to be less toxic than metaphen, which contains the same amount of mercury (Birkhaug, 1930). The chief objection to the use of this disinfectant is its insolubility in water, a saturated solution containing only 0.79 gramme per litre.

Phenyl mercuric acetate was tried because it was more soluble and would appear to be effective in a concentration of 1/100,000; but this concentration precipitated serum proteins.

Proflavine 1/100,000 prevented growth of *Staph. aureus* and *B. subtilis* for six days and enterococcus for three days. A concentration of 1/10,000 did not prevent the growth of moulds or of *Ps. pyocyanea*. Eggerth's (1926) observations on the effect of the hydrogen-ion concentration could not be repeated; proflavine was as active in plasma at a pH of 6.9 as in serum at a pH of 8.4.

Sulphanilamide proved ineffective in serum and plasma in inhibiting bacterial growth. Novak (1939) and Hunwicke (1940) have reported that 1/5,000 sulphanilamide would prevent the growth of organisms in stored citrated blood for twenty-one days. In serum from an inoculum of 20 organisms per c.cm. there is no growth of *Staph. aureus*, *B. subtilis*, *M. tetragenus*, or enterococci for twenty-four hours after incubation at 37° C.; but after this time growth of all these organisms was observed and the degree of inhibition depended on the organisms. Enterococci and micrococci grew readily at 37° C., the count of micrococci increasing from 4,150 bacteria per c.cm. in two days to 53,600,000 in seven days. Control counts on serum without sulphanilamide during the same period showed a count of 466,500,000 in two days and 54,500,000 in seven days. At 4° C. serum which had become contaminated with *M. tetragenus* and had an initial count of 200 organisms per c.cm., after three days in the presence of sulphanilamide contained 5,500 organisms per c.cm., and after six days contained 45,000 bacteria per c.cm. Counts on serum without sulphanilamide increased from 4,350 bacteria per c.cm. in three days to 45,000 in six days. This serum had been held for some months at -20° C. Growth of *Staph. aureus* was inhibited to some extent by sulphanilamide. In serum inoculated with 20 organisms per c.cm. and incubated at 37° C. the count after two days was 24,000,000 organisms per c.cm. and 91,500,000 after seven days. Serum containing 1/5,000 sulphanilamide under similar conditions had a bacterial count of 9,000 bacteria per c.cm. in two days and 2,900,000 in seven days. It would appear, therefore, that sulphanilamide will inhibit the growth of organisms in serum for twenty-four hours but cannot be relied on for any longer period.

The results obtained when using citrated whole blood as a growth medium for the bacteria substantiate those previously described by Novak in human blood and by Hunwicke in horse and guinea-pig blood.

Thymol and *p-chlor-m-cresol* were shown to be quite unsuitable as serum preservatives: both allowed the growth of organisms in a dilution of 1/2,000, and caused the precipitation of serum protein.

Discussion

From these experiments it would appear that disinfectants can have only a limited value in controlling the bacterial growth in stored serum. Of those tried the most effective was proflavine. In a concentration of 1/100,000 it prevented the growth of *Staph. aureus* and *B. subtilis* for six days and of enterococcus for three days. A dilution of 1/10,000 had no effect on *Ps. pyocyanea* or mould. From recent work (Albert *et al.*, 1938) it is evident that much higher concentrations are equally ineffective against pseudomonas. Higher concentrations of any of these disinfectants would not be practicable because of the danger of toxic reactions.

Bottles of serum sent by the Emergency Blood Supply Depots of the London region for drying in this laboratory

have been tested for sterility as they were received, and the results of these tests have been reported back to the depots. Some of the bottles have been found to be contaminated, and the number has steadily decreased as the depots have gained experience in the separation of the serum. As in no case was there any evidence of gross infection, and as the bottles are held at 4° C., at which temperature bacterial growth is slight, the infected bottles were pooled with the sterile ones during filtration. The majority of the contaminants were spore-bearing aerobes of the *subtilis* type, and Gram-positive cocci—either micrococci or sarcinae; pseudomonas was also isolated from serum and from stored citrated plasma. The necessity for filtration is evident, and from experimental results it is also evident that, although the contaminants of the *subtilis* type would be susceptible to the action of disinfectant, the Gram-positive cocci and pseudomonas would be resistant to its action, and the value of adding disinfectant to serum is doubtful.

While it is obvious that the correct procedure is to maintain conditions of absolute sterility during both the preparation and the solution of dried serum, and that the reconstituted serum should be used immediately, it is possible that in wartime reconstitution might take place under difficult conditions and it might not be possible to use the serum immediately. In such circumstances results would indicate that proflavine 1/100,000 and phenyl mercuric nitrate 1/50,000 would prevent bacterial growth for three days.

Summary

The effect of various disinfectants in controlling the growth of bacteria in serum, dried and reconstituted serum, and citrate plasma has been studied.

Growth of *B. subtilis* and *Staph. aureus* was prevented for six days by merthiolate 1/10,000, metaphen 1/10,000, phenyl mercuric nitrate 1/50,000, and proflavine 1/100,000. Growth occurred in sulphanilamide 1/5,000 after twenty-four hours.

Enterococcus was inhibited for three days by phenyl mercuric acetate 1/100,000, proflavine 1/100,000, and merthiolate 1/10,000. Growth in three days could be demonstrated in the presence of metaphen 1/10,000, phenyl mercuric nitrate 1/50,000, and sulphanilamide 1/5,000.

Ps. pyocyanea was not inhibited by any of these disinfectants.

Thymol and *p*-chlor-*m*-cresol were ineffective as bacteriostatics; and these and phenyl mercuric acetate precipitated proteins in serum.

Freezing at -20° C. and drying in serum did not affect the bacteriostatic power of metaphen, sulphanilamide, proflavine, and phenyl mercuric nitrate; nor did the presence of the disinfectants affect the solubility of the dried serum.

Moulds were inhibited by 1/50,000 phenyl mercuric nitrate but not by 1/10,000 proflavine.

Sulphanilamide will prevent the growth of *B. subtilis*, *M. tetragenus*, and pseudomonas in whole blood for thirty days at 4° C. but not at 22° C.

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The Foreign Department of the Reich Students' Administration announces (*Klin. Wsch.*, 1940, **19**, 1296) that more than 3,300 foreign students of both sexes are now enrolled in German universities. It is stated that no fewer than 65 countries of the world have sent delegates from their student youth to study in Germany.

LACTOSE FOR PREVENTION OF ODOUR IN THE CLOSED-CAST TREATMENT OF COMPOUND FRACTURES

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The increasing use of the closed-cast method of treating compound fractures, particularly under wartime conditions, induces us to propose a means of preventing the foul odour that is one of its principal disadvantages. Our procedure, of which a more detailed account will be published elsewhere, has been devised in connexion with the treatment of osteomyelitis by the Winnett Orr technique. The application of that method to compound fractures has been well described by Trueta (1939).

Protein-sparing Effect of Carbohydrate

Briefly stated, it is our hypothesis that the foul odour which develops in these casts is the result of putrefaction, defined by Rettger (1928) as "anaerobic decomposition of protein with the formation of foul-smelling products characteristic of cadaveric decomposition." Autolysis of devitalized tissue probably helps to initiate this proteolysis by providing preliminary breakdown products which are more readily attacked by micro-organisms than are native tissue proteins. It seems likely that anaerobic bacteria play an important part in putrefaction. Trueta (1939) has noted the presence of anaerobes in the wounds under consideration. Whether these organisms arrive through the blood stream or by accidental implantation remains to be determined. On the basis of these assumptions, by implanting a sugar in the wound one may take advantage of the fact that most bacteria will attack carbohydrate in preference to protein when both are simultaneously available. As Kendall *et al.* (1913) have expressed it: "Fermentation takes precedence over putrefaction." It is when bacteria are utilizing protein for fuel rather than for merely structural requirements that foul products are formed. This principle is commonly referred to as the "protein-sparing effect of carbohydrate."

In selecting a sugar for use in wounds lactose was chosen instead of glucose because, since its molecule is larger (Kendall and others, 1913), a solution of it isotonic with the blood contains more sugar, and (Kendall, 1923) loss by absorption from the wound should be less. Since the first stage in the fermentation of lactose is the production of galactose and glucose, the latter becomes available for the relatively few organisms which cannot utilize lactose.

Method of Using Lactose

For clinical use we have arbitrarily chosen a 12% solution. Lactose of U.S.P. grade is dissolved in distilled water and autoclaved in quantities of about 50 c.cm. Sterile gauze dressings which have been dipped in this solution are placed in the wound instead of the usual vaselined gauze packs. A covering of dry gauze is employed to keep the sugar from contact with the plaster lest crumbling occur. In débridement the solution should be made and autoclaved within two hours of the time the water is distilled, as a precaution against the presence of spores. At subsequent re-dressings this precaution is unnecessary, since the granulation tissue is an adequate barrier against toxins (White, 1931).

We have employed this method in Winnett Orr casts for periods up to four weeks without the development of