

Effects of Surgical Antiseptics on Streptofibrinolysin.*

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To determine the effects of surgical antiseptics on the fibrinolytic activity of *Streptococcus hemolyticus* tests were made of: (a) the effects of sub-bacteriostatic concentrations of the commoner antiseptics on the rate of fibrinolysin-production by glucose veal-infusion broth cultures of *S. hemolyticus*, and (b) the effects of certain selected concentrations of the same antiseptics on the coagulation and autolysis of human fibrin and on the liquefaction of isolated human fibrin by streptococcal cultures (or filtrates).

To determine the effects of antiseptics on fibrinolysin-production sub-bacteriostatic dilutions of the commoner antiseptics (Table I) were made in routine dextrose veal-infusion broth. Ten cc. of each of the resulting antiseptic-broth mixtures were inoculated with one loopful of a 24-hour broth culture of *S. hemolyticus* and incubated at 37°C. for 24 hours. The total bacterial population and the total number of fibrinolytic units per cc. were then determined for each subculture. (For technics see previous paper.¹) Typical data are recorded in Fig. 1.

The table shows a partial inhibition of lysin-production in sub-bacteriostatic concentrations of zonite, the lysin-production varying proportionally with the rate of streptococcal proliferation. The same parallelism between bacterial count and lytic titer was noted with all other antiseptics (Table I) thus far tested by us. This parallelism tends to confirm our previous conclusion that there is a quantitative linkage between the rate of cell-division and the rate of fibrinolysin-production with *S. hemolyticus*.

To study the effects of the same antiseptics on coagulation and autolysis of human fibrin, solutions of fibrinogen and thrombin were prepared by the technic of Tillett and Garner.² Serial dilutions of the stock antiseptic solutions were made in the standard fibrino-

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¹ Madison, R. R., and Taranik, Jeannette D., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 1.

² Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

TABLE I.

Effects of Antiseptics on Coagulation and Autolysis of Human Fibrin on Its Susceptibility to Streptofibrinolysin.

The first column records the highest dilution of each stock-solution that prevents *in vitro* coagulation of human fibrinogen-thrombin complex. Blank (—), no inhibition of clotting even with a 1:1 dilution of the antiseptic solution.

The second column records the highest dilution of the same stock antiseptic that will prevent subsequent autolysis. Blank (—), no inhibition of autolysis, even with a 1:1 dilution of the stock antiseptic.

The third and fourth columns record the arbitrary dilutions tested for their possible denaturing effects on human fibrin, and the percentage susceptibility of the resulting antiseptic fibrin complex.

| Stock Solution of Antiseptic | Highest dilution | | Effect on fibrinolysis | |
|------------------------------------|---------------------|----------------------|------------------------|-----------------------|
| | Inhibiting clotting | Preventing autolysis | Critical dilution | Fibrin susceptibility |
| 0.8% NaCl-solution (control) | — | — | — | 100 |
| 0.1% Zonite | 1:30 | 1:300 | 1:300 | 100 |
| 2% Phenol | — | — | 1:3 | 100 |
| 1% Mercuric chloride | 1:30 | — | 1:300 | 100 |
| 1% Merthiolate | 1:30 | — | 1:300 | 100 |
| 1% Mercurochrome | 1:30 | — | 1:300 | 100 |
| 0.6% Potassium permanganate | 1:30 | — | 1:300 | 100 |
| 0.5% Metaphen | 1:3 | — | 1:30 | 100 |
| Mercresin* | 1:3 | — | 1:30 | 100 |
| 0.03% Phenyl mercuric nitrate | — | — | 1:3 | 100 |
| Mi 31* | 1:3 | — | 1:30 | 100 |
| 0.5% Hexyl resoreinol | stock | — | 1:30 | 100 |
| 1.22% Picric acid | 1:30 | — | 1:300 | 100 |
| 100% Glycerin | — | 1:3 | 1:30 | 100 |
| 0.1% Acriflavine | 1:3 | 1:30 | 1:30 | 100 |
| 97% Ethyl alcohol | 1:3 | — | 1:30 | 100 |
| 5.15% Boric acid | 1:3 | 1:300 | 1:30 | 100 |
| 2% Tannic acid | 1:300 | — | 1:3000 | 30 |
| Sulpho-salicylic acid (sat. solu.) | 1:300 | — | 1:3000 | 30 |
| 0.1% Gentian violet | 1:3 | 1:30 | 1:30 | 30 |
| 5.25% Hypochlorite | 1:300 | — | 1:3000 | 20 |
| 2% Lysol | 1:3 | — | 1:30 | 19 |
| Tincture iodine | 1:300 | — | 1:3000 | 12.5 |
| 0.029% Iodine in water | 1:3 | — | 1:30 | 12.5 |
| 40% Formaldehyde | 1:3 | 1:30 | 1:30 | 3 |
| 2.5% Prontosil | — | — | 1:3 | 100 |
| 10% Sulphonamide | 1:30 | — | 1:300 | 100 |
| 10% Thromboplastin* | — | — | 1:3 | 100 |

*Standard commercial product.

gen-solution and coagulation attempted by the addition of a routine dose of thrombin. The maximal dilution of each antiseptic that prevents coagulation by this technic is recorded in the first column of Table I.

Antiseptic-free human fibrin prepared by this technic usually undergoes spontaneous autolysis within 24 hours at 37.5°C. Several of the antiseptics prevented this autolysis. The maximal inhibiting titers for these antiseptics are recorded in the second column of Table I.

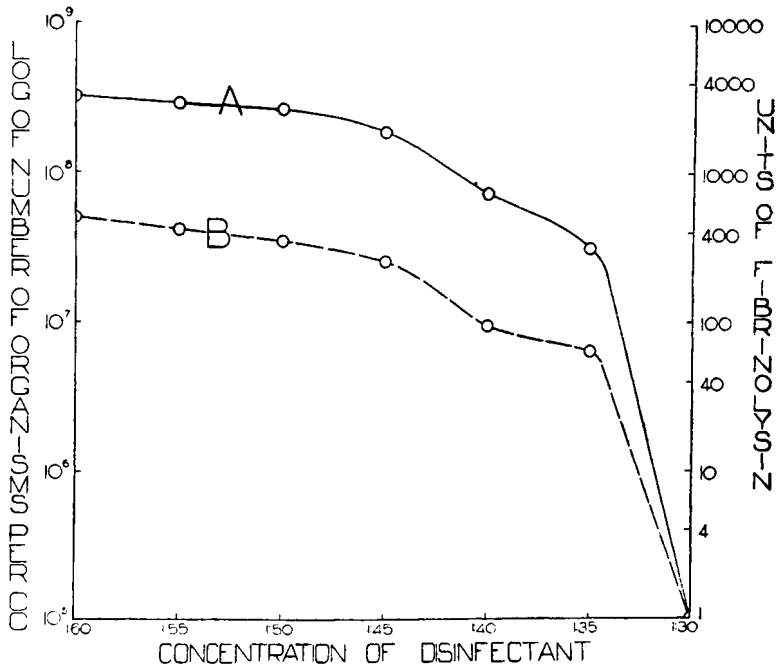


FIG. 1.

Typical Effects of Dilute Antiseptics on Streptococcal Proliferation and Fibrinolysin Secretion.

Data obtained with a 0.1% stock solution of zonite. 1:60 to 1:30 serial dilutions of this stock antiseptic in glucose, veal-infusion broth, inoculated with a standard loopful of a 24-hour broth culture of *S. hemolyticus*, and incubated at 37.5°C. for 24 hours.

A, Total number of streptococci per cc. in each 24-hour sub-culture, as determined by the Petroff-Hausser counting chamber.

B, Total number of fibrinolytic units per cc. in the same sub-cultures. (For a definition of the arbitrary fibrinolytic unit see previous papers.¹)

Possible chemical denaturation of human fibrin (or possible inhibition of the lytic enzyme) was tested with certain selected dilutions of each antiseptic. Data thus obtained are recorded as the relative susceptibility of the resulting fibrin-antiseptic complex to routine doses of streptofibrinolysin. Thus, if in a control tube of antiseptic-free human fibrin liquefaction is observed by the end of 2 hours as a result of the addition of a 1:500 dilution of a given streptococcal culture (or filtrate), while in the presence of the selected antiseptic, a 1:50 dilution of the same culture (or filtrate) is required, the antiseptic-fibrin complex is apparently but 10% as susceptible as normal human fibrin to streptofibrinolysin. Data thus calculated are recorded in the fourth column of Table I.

According to Menkin,³ and others the main factor preventing the

³ Menkin, V., *J. Exp. Med.*, 1931, **53**, 647; 1932, **56**, 157; 1933, **57**, 977.

spread of staphylococci, streptococci and related microorganisms from a local focus is the meshwork of fibrin filling interstitial tissue-spaces and plugging regional lymphatics. If this is true, antiseptics which render encapsulating deposits of fibrin relatively unsusceptible to streptofibrinolysins would be antiseptics of choice in the local treatment of streptococcal infections. In our hands tincture of iodine is more efficient in reducing fibrinolytic susceptibility than many of the newer antiseptics, a 1:3000 dilution of the tincture rendering fibrin but one-eighth its normal susceptibility to liquefaction by streptococci.

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Ultrafiltration of Virus of Equine Encephalomyelitis (Russian Strain, Moscow No. 2)

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Bauer, Cox and Olitsky¹ have recently determined the filtration end-point of the Eastern and Western strains of the virus of equine encephalomyelitis. Their results have indicated a particle size of 20 to 30 m μ for both types. Previous work by one of us² has indicated the immunological independence of a Russian strain (Moscow No. 2) of this virus, and it was considered of interest to compare its size with the results already reported for the two North American strains.

A 20% suspension of guinea pig brain containing active virus was prepared in a diluting fluid consisting of equal parts of hormone broth, ascitic fluid and sterile distilled water as recommended by Bauer, Cox and Olitsky.¹ A 1-100 dilution of the stock suspension in the same fluid was used for the ultrafiltration experiments, while all titrations of the virus whether before or after filtration were regularly made in the same diluent.

The collodion membranes were prepared according to the method of Elford,³ as modified by Bauer and Hughes.⁴ All filtrations were

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¹ Bauer, J. H., Cox, H. R., and Olitsky, P. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 378.

² Howitt, B. F., *J. Immun.*, 1935, **29**, 319.

³ Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

⁴ Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.