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## Failure of Sulfanilamide to Inactivate Preformed Hemotoxins, Diphtheric Toxin, or Tetanal Toxin.

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Experiments on the action of sulfanilamide on *beta* hemolytic-streptococcal infections in cultures of human marrow[1] indicated that sulfanilamide, in some manner, interfered with the production of the toxins responsible for the virulence of the organism and for its resistance to small amounts of bactericidins. To determine whether sulfanilamide would inactivate preformed toxins or affect other toxins, the experiments herein described were performed.

Beta hemolytic streptococci, strain AB-13, were grown in Hartley broth for 16 to 24 hours and the Seitz filtrate was tested for hemotoxin with rabbit, human, and sheep cells. No hemotoxin was demonstrable even when the filtration was carried out in an atmosphere of nitrogen and reducing agents were added to the filtrate. Therefore, unfiltered 16- to 24-hour cultures were diluted with saline or saline plus sulfanilamide, and incubated for one hour in a 37.5° waterbath, with equal volumes of a 1% saline suspension of rabbit, human, or sheep cells. The concentrations of sulfanilamide used were 1-1000, 1-10,000, and 1-100,000. When this technic was employed, complete hemolysis occurred in all tubes containing sulfanilamide corresponding in dilution to the controls which showed complete hemolysis. However, hemolysis became complete from 1 to 15 minutes earlier in the controls than in the corresponding dilutions containing sulfanilamide. Since this might be explained by bacteriostasis, similar experiments were performed in which 0.1 volume of 1-1000 aqueous merthiolate was added to each tube to prevent further bacterial growth. With this precaution no differences were noted between the tubes containing sulfanilamide and the corresponding controls. If the hemolytic streptococci were inoculated in Hartley broth, the cultures divided into 2 equal parts and sulfanilamide added to one, the production of hemotoxin was delayed but not prevented in the culture containing sulfanilamide.

[1] Osgood, E. E., *J. A. M. A.*, 1938, **110**, 349; Osgood, E. E., and Brownlee, Inez E., *J. A. M. A.*, 1938, **110**, 1770.

Hemotoxin* from the hemolytic *Staphylococcus aureus* was not inactivated by sulfanilamide in concentrations up to 1-1000, nor was a sample of *B. perfringens* hemotoxin.†

Hemotoxins were prepared as filtrates of *Cl. tetani* (48-hour), *Cl. oedematis-maligni* (16-hour), and *B. perfringens* (16-hour) broth cultures, and as filtrates of hemolytic *Staphylococcus aureus* (48-hour) 0.2% semisolid agar cultures.‡ Incubation at 32°C seemed to favor especially the production of tetanal hemotoxin. The minimal hemolytic doses varied from 1/8 to 1/64 cc for an amount of washed cells equivalent to 1/40 cc of whole blood. Human, sheep, and rabbit erythrocytes were not hemolyzed to the same extent. The data herewith given relate to human cells.

Attempts were made to inactivate the various hemotoxins with sulfanilamide. To each tube of a series of progressively halved concentrations of sulfanilamide, 1-500 to 1-8,192,000, were added one minimal hemolytic dose of hemotoxin, and one test-dose of erythrocytes; final volume, 1.0 cc. Incubation was conducted for one hour at 37°C, proper control tubes being included.

No inactivation of the hemotoxins of *Cl. tetani, Cl. oedematis-maligni,* or hemolytic *Staphylococcus aureus* was demonstrable by the above method. A variation in method comprising 30 minutes' contact of hemotoxin and sulfanilamide in the icebox, followed by the addition of erythrocytes and regular incubation caused no inactivation of these 3 hemotoxins. On the other hand, sulfanilamide gave what appeared to be partial inactivation of the *B. perfringens* hemotoxin; the degree of hemolysis being 2 plus with all concentrations of sulfanilamide down to and including 1-4,096,000. However, with additional incubation of an hour or two at room temperature, hemolysis became complete. The reason for this slowing of hemolysis, simulating inactivation of one of two or more hemotoxins by antitoxin, has not yet been determined, although it was noted in hemotoxin aged up to 10 days but not 15 days or over.

Experiments were conducted in which various concentrations of sulfanilamide were incorporated in *B. perfringens* culture-broths in which hemotoxin was to be produced. It was found that concentrations of sulfanilamide of 1-10,000, 1-20,000, and 1-50,000 did not interfere with the production of hemotoxin or growth of the culture

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† Supplied by Eli Lilly and Company, Indianapolis, Indiana.

‡ All of the experiments on hemotoxins were performed in the Biological Research Division, Eli Lilly and Company, Indianapolis, Ind.

as compared to control cultures without sulfanilamide. All 4 of these samples of hemotoxin had an MHD of 1/64 cc.

Saponin in a dose of 0.5 cc of a 1-4000 dilution was fully hemolytic for our test-dose of erythrocytes. This hemotoxin was observed, as is well known, to be enhanced in potency by benzol, the increase being 8-fold when 1-10,000 benzol was used. Sulfanilamide, however, did not affect the hemolytic action of saponin.

We have attempted without success to cure guinea pigs treated with diphtheric or tetanal toxins. With these toxins, *in vivo* tests

TABLE I.
Effects of Sulfanilamide on Survival of Guinea Pigs Given Diphtheric Toxin.

| Controls | | | Sulfanilamide-Therapy | | | |
|---|---|---|---|---|---|---|
| No. of pigs | MLD | Hr survival | No. of pigs | MLD | Dose of sulfanilamide | Hr survival |
| | | | | | mg | |
| 1 | 100 | 17 | 1 | 100 | 2 initial | 17 |
| 1 | 50 | 17 | 1 | 50 | 2 " | 17 |
| 1 | 25 | 17 | 1 | 25 | 2 " | 17 |
| 2* | 10 | <96 | 2* | 10 | 50 at 1 hr<br>50 " 5 " | <96 |
| 2* | 5 | <96 | 2* | 5 | 50 " 1 "<br>50 " 5 " | <96 |
| 2 | 2.5 | 21.5, 22 | 2 | 2.5 | 2 initial<br>2 q. 4 h. | 21.5, 22 |
| 2 | 1.25 | 19.5, 84 | 2 | 1.25 | 2 initial<br>5 q. 4 h. | 41.5, 72 |
| 4* | 1 | <96 | 4* | 1 | 50 at 1 hr<br>50 " 5 " | <96 |

* Performed in Indianapolis.

TABLE II.
Effects of Sulfanilamide on Survival of Guinea Pigs Given Tetanal Toxin.

| Controls | | | Sulfanilamide-Therapy | | | |
|---|---|---|---|---|---|---|
| No. of pigs | MLD | Hr survival | No. of pigs | MLD | Dose of sulfanilamide | Hr survival |
| | | | | | mg | |
| 5 | 105 | 25, 28, 30, 32, 32 | 5 | 105 | 5 q. 4 h. | 33, 34, 34, 41.5, 44 |
| 3 | 60 | 28, 31, 40 | 3 | 60 | 5 q. 4 h. | 38, 45, 45 |
| 12 | 30 | 2 lived, 36, 36, 36, 40, 40, 43, 48, 50, 50, 53 | 12 | 30 | 5 q. 4 h. | 5 lived, 35, 35, 41, 46, 48, 50, 50 |
| 1 | 12 | lived | 1 | 12 | 2 q. 4 h. | lived |
| 2* | 10 | <96 | 2* | 10 | 75 at 1 hr<br>75 " 5 " | <96 |
| 2* | 5 | <96 | 2* | 5 | 75 " 1 "<br>75 " 5 " | <96 |
| 1 | 3.5 | lived | 1 | 3.5 | 2 q. 4 h. | lived |
| 1 | 1.5 | " | 1 | 1.5 | 2 q. 4 h. | " |
| 4* | 1 | <96 | 4* | 1 | 75 at 1 hr<br>75 " 5 " | <96 |

* Performed in Indianapolis.

obviously could yield more important results than could *in vitro* tests. It may be mentioned that diphtheric toxin is devoid of hemotoxin, while tetanal toxin as usually produced (by incubation of the culture for about 2 weeks) is also devoid of hemotoxin but contains considerable hemotoxoid.

It will be noted (Tables I and II) that sulfanilamide-therapy had absolutely no effect on the survival of guinea pigs receiving diphtheric toxin, and very little effect on those receiving tetanal toxin. It seems probable that in the experiments with tetanal toxin the slight differences in favor of the guinea pigs receiving sulfanilamide may be explained by chance variation.

*Conclusion.* Sulfanilamide in concentrations of 1-1000 or less does not inactivate *in vitro* significant amounts of the hemotoxins of the *beta* hemolytic *streptococcus,* hemolytic *staphylococcus aureus, Cl.oedematis-maligni, Cl. tetani,* or *B. perfringens.* Sulfanilamide-therapy did not significantly affect the course of intoxication in guinea pigs initiated with as little as 1 MLD of diphtheric or tetanal toxin.

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## Preparation of Extracts of the Renal Pressor Substance.*

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During recent years, evidence has accumulated which suggests that elevation of blood pressure may be produced by some substance or substances which occur in renal tissue. In the course of investigations on the relationship of certain types of experimental hypertension to this renal pressor substance, one difficulty frequently encountered has been that of obtaining uniform and potent renal extracts. An attempt was made to determine the factors which affect the yield of this pressor substance, and to develop a standardized method of preparation.

Fresh kidneys of hogs were obtained from the slaughter house. The kidney cortex was dissected with scissors from the medulla, and the cortical tissue passed through a meat grinder. The minced kidney cortex was ground to a fine paste with carborundum. The possibility that carborundum might adsorb some of the pressor substance was

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