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Inactivation of Toxins of *Staphylococcus aureus* and *Clostridium welchii in vitro* by Sulfanilamide.*

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Recently, we[1,2] demonstrated that sulfanilamide prevents the death of mice which have been injected intraäbdominally with lethal amounts of gonococcal "toxin", and that this "toxin" can be inactivated *in vitro* by exposure to the drug for several hours at 37°C. It was later observed[3] that a toxin obtained from a single strain of *Staphylococcus aureus* became non-toxic immediately after contact with sulfanilamide *in vitro*. The present paper records the results of a similar study with the toxins of other strains of *Staphylococcus aureus* and the toxin of *Clostridium welchii*. Osgood and Powell[4] have reported, "Sulfanilamide in concentrations of 1:1,000 or less does not inactivate *in vitro* significant amounts of the hemotoxins of . . . . hemolytic *Staphylococcus aureus* . . . . or *B. perfringens*." This finding is not in accord with the results which we have obtained during the last year.

The toxins from 3 hemolytic strains of *Staphylococcus aureus* were investigated. Two of the strains were recovered in our laboratories from purulent discharges, one from otitis media and the other from a furuncle. The third strain, received from Dr. C. E. Dolman, was isolated from the nasal mucosa of a patient with small boils in and about the nose. The different lots of toxin were prepared according to the technic of Dolman.[5] A 36-hour culture of the organism grown on semi-solid agar was combined with Douglas' broth and filtered through cheese-cloth and filter paper. It was then centrifugalized, and the supernate, which constituted the toxin, was removed. Merthiolate was added to give a final concentration of 1:8,000.

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1 Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530.

2 Carpenter, C. M., Barbour, G. M., and Hawley, P. L., *J. Bact.*, 1938, **36**, 280.

3 Carpenter, C. M., Barbour, G. M., and Hawley, P. L., *J. Pediatrics*, 1939, **14**, 116.

4 Osgood, E. E., and Powell, H. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 37.

5 Dolman, C. E., *Canad. Public Health J.*, 1932, **23**, 125.

The MLD of each lot of toxin was determined for 20 g Rockland mice and was found to range from 0.1 to 0.2 cc. Sulfanilamide (in 0.85% sodium-chloride solution) in dilutions of from 1:33 to 1:1,000 was mixed with the toxin in a ratio of 1 cc of the dilution of the drug employed to 1 MLD of the toxin. The mixture was shaken and immediately injected intraäbdominally into mice. A total of 825 mice was injected, 195 of which were controls. From 89 to 97% of 630 mice receiving the "inactivated" toxin lived, the percentage varying inversely with the concentration of the sulfanilamide used for inactivation. None of the 195 control mice survived (Table I). The work extended over a period of several months, during which time several different lots of toxin were used.

The inactivation of staphylococcal toxin by sulfanilamide *in vitro* prompted an investigation of the *in vivo* effects of the drug. The compound was injected intraäbdominally into mice in doses ranging from 3 to 30 mg from 15 minutes to 3 hours prior to the intraäbdominal administration of 1 MLD of the toxin. In this experiment, 144, or 60%, of 240 mice survived. When a single dose of 30 mg of the drug was similarly injected immediately after the administration of the toxin, 16, or 27% of 60 mice lived. Only 16, or 10% of 160 mice survived when sulfanilamide was administered in 2 doses, 10 mg immediately after the toxin and 20 mg 5 hours later.

Employing the procedure outlined for the *in vitro* inactivation of staphylococcal toxin by sulfanilamide, a similar study was made of the action of the drug on the toxin of *Clostridium welchii*.† The MLD for 20 g mice was determined to be 0.08 cc. An area of necrosis

TABLE I.
Intraäbdominal Injection of Mice with Staphylococcal Toxin Inactivated *in Vitro* by Sulfanilamide.

Group	Dil. of sulfanilamide in 0.85% NaCl solution	Mice injected with toxin-sulfanilamide mixture			Mice injected with toxin only		Difference*
		No. injected	No. survived	% survived	No. injected	No. survived	S.E.D.M.
1	1:33	120	107	89.2	30	0	9.6
2	1:50	180	160	88.9	45	0	11.7
3	1:66	100	89	89.0	25	0	8.8
4	1:100	60	57	95.0	20	0	8.4
5	1:500	60	58	96.6	20	0	8.9
6	1:1000	110	107	97.3	55	0	13.5
Total		630	578	91.7	195	0	24.5

* Difference ÷ standard error of the difference of the means. The odds against the findings being due to chance approach infinity.

† The toxin of *Clostridium welchii* (perfringens-044692-B) was supplied by Parke, Davis & Company.

TABLE II.
Intraäbdominal Injection of Mice with the Toxin of *Clostridium welchii* Inactivated *in Vitro* by Sulfanilamide.

Group	Dil. of sulfanilamide in 0.85% NaCl solution	Mice injected with toxin-sulf. mixture		Mice injected with toxin only		Difference*
		No. injected	No. survived	No. injected	No. survived	S.E.D.M.
1	1:33	20	15	5	0	
2	1:100	20	16	5	1	
3	1:500	20	15	5	1	
4	1:500	20	18	5	0	
5	1:1000	20	19	5	0	
6	1:1000	20	18	5	0	
Total		120	101 (84.2%)	30	2 (6.7%)	8.2

*Difference ÷ standard error of the difference of the means. The odds against the findings being due to chance approach infinity.

always developed at the site of injection. Findings at autopsy were usually generalized edema, subcutaneous hemorrhage, and focal areas of necrosis in the kidneys and liver. The toxin was mixed *in vitro* with the same dilutions of sulfanilamide as those used to inactivate the staphylococcal toxin. The mixture was injected at once, either intraäbdominally or intramuscularly into mice. Eighty-four percent of 120 mice injected intraäbdominally (Table II) and 86% of 140 mice injected intramuscularly survived. One MLD of the toxin, when injected intraäbdominally, killed 93% of 30 control mice within 5 days. When the toxin was injected intramuscularly into the thigh, 89% of 30 control mice died within 7 days.

The *in vitro* inactivation of the toxin of *Clostridium welchii* was followed by a study of the therapeutic effect of sulfanilamide on mice injected with the toxin. It was found that when 30 mg of sulfanilamide in one cc of 0.85% sodium chloride solution were administered intraäbdominally within 60 hours following the intraäbdominal injection of 1 MLD of the toxin, 100, or 83% of 120 mice survived, while 90% of 30 untreated control mice died within 5 days. When the compound was given within 60 hours after intramuscular injection of the toxin, 108, or 90% of 120 mice were protected, while 87% of 30 untreated control mice died within 7 days.

The nature of the toxin-sulfanilamide reaction has not been determined. It is at present under study. The fact that the toxins, neutralized by exposure to sulfanilamide, regain their toxicity after various periods of standing at room temperature, indicates that the reaction is, in part at least, reversible.

Summary. Toxins from 3 hemolytic strains of *Staphylococcus aureus* were inactivated by sulfanilamide *in vitro*. This was demon-

strated by the survival of 92% of 630 mice injected intraäbdominally with the sulfanilamide-toxin mixture and the death of all 195 controls which received toxin only. The *in vivo* action of sulfanilamide, administered either before or subsequent to the toxin, was less marked.

The toxin of *Clostridium welchii* was likewise inactivated *in vitro* by sulfanilamide. Eighty-four per cent of 120 and 86% of 140 mice, injected intraäbdominally and intramuscularly, respectively, with the toxin-sulfanilamide mixture survived. *In vivo,* sulfanilamide protected 83% of 120 mice, when administered after the intraäbdominal injection of the toxin, and 90% of 120 mice after intramuscular inoculation. Only 8% of 60 control mice, injected intraäbdominally, and 12% of 60 control mice, injected intramuscularly, lived.

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Effect of Petroleum Ether Extract of Mouse Carcasses as Solvent in Production of Sarcoma.*

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The production of sarcomata in mice may be influenced by the solvent used for the carcinogenic hydrocarbon.[1] Most experiments have been carried out with vegetable oils, lard, cholesterol or paraffin which are effective vehicles for 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and methylcholanthrene. The increase in the incidence of skin tumors in mice following the application of mouse fat to the skin before tarring, as reported by Watson and Mellanby,[2] led us to investigate the effect of a petroleum ether extract of mouse tissues as a solvent for 3:4-benzpyrene in the production of connective tissue sarcoma.

The extract was prepared by refluxing fresh minced mouse carcasses, from which the stomach and intestines had been excised, with petroleum ether (maximum boiling point 50°C) for 16 hours. The petroleum ether was removed by distillation under reduced pressure.

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1 Peacock, P. R., and Beck, S., *Brit. J. Exp. Path.*, 1938, **19**, 315.

2 Watson, A. F., and Mellanby, E., *Brit. J. Exp. Path.*, 1930, **11**, 311.