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Experimental Streptococcal Infections in Animals for Therapeutic Investigations. I. Virulence-tests.

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The recent introduction of sulfanilamide and sulfapyridine in the treatment of *Streptococcus hemolyticus* infections has aroused a great deal of interest. It was felt that a further study of the effect of the drug in experimental hemolytic streptococcal infections produced in animals would be highly desirable. It is recognized that experimental work on *Streptococcus hemolyticus* has been handicapped by the lack of a susceptible laboratory animal. Although there are a few virulent strains which can kill mice at a dilution of 10^{-6} or 10^{-7} when injected intraäbdominally, their value is somewhat limited for several reasons. (1) As Kolmer[1] has pointed out a highly virulent strain produces fatal infection too rapidly for therapeutic investigations, since the severity of the infection usually does not permit the administration of repeated doses of the agent under study and may mask any slight curative activity which is insufficient to produce a complete disinfection in one or 2 doses at daily intervals. (2) A drug which works well in the virulent strains may not do the same in all strains of *Streptococcus hemolyticus* infections. (3) It is highly desirable to compare the virulence of the organisms isolated from different patients. At the outset of our study it was found that the strains of hemolytic streptococci obtained locally from human sources were relatively avirulent for laboratory animals. It was necessary to employ a relatively large dose, namely, 0.1 to 0.05 cc of an 18-hour serum-broth culture, to kill a mouse when injected intraäbdominally. A few attempts made to increase the virulence of some of our strains of hemolytic streptococci by repeated passages through mice were not successful. This finding rendered our proposed study difficult, since protection-tests conducted with large infecting doses of organisms are in general not very satisfactory.

Kolmer[1] introduced the endermal infection in rabbit and observed the rate of healing of the lesion for the therapeutic investigation. This requires the use of a large animal and a moderately virulent

[1] Kolmer, J. A., and Rule, A. M., *J. Lab. Clin. Med.*, 1937, **22**, 1097.

strain *i.e.* a strain that will kill a mouse with 0.001 cc of an 18-hour broth culture when given intraäbdominally. The strains isolated locally were not so virulent. Miller[2] was able to enhance the virulence of meningococcus for mice by the use of mucin. A similar procedure tried by us with hemolytic streptococci has so far yielded negative results. Norton and Dingle[3] have recently shown that intracerebral injections of *E. typhi* in mice resulted in an increase in the lethal effect of the organism for the animal when compared with intraäbdominal injections. This method was tried with several strains of hemolytic streptococci isolated recently from our cases of scarlet fever and erysipelas and was found to be satisfactory. So the aim of this study is to compare the lethal effects of *Streptococcus hemolyticus* for small laboratory animals when the organisms are introduced intraäbdominally, interpleurally, intravenously, and intracerebrally.

Experiments. Because of the shortage of white mice at the time when this study was started, Chinese hamsters of 15-20 g were employed at first. Two strains of *Streptococcus hemolyticus* were used, namely, SA_{17} and E_1 which were isolated recently from patients suffering from scarlet fever and erysipelas respectively. The amount given intraäbdominally and intrapleurally was 0.5 cc of the desired dilution of the organism, while that given by the intracerebral route was 0.05 cc. Animals receiving interpleural or intracerebral injections were previously anesthetized with ether. The organism used was an 18-hour culture in a 5% rabbit-serum, 0.1% dextrose, meat-infusion broth. For each route of inoculation 5 different dilutions of organisms were given and for each dilution 3 animals were used. Animals were kept for 3 weeks before they were discarded. The result is shown in Table 1. All the animals were necropsied after death. Direct smears and cultures were made from the places of inoculation and also from the heart blood. In all animals the direct smears and cultures from the places of inoculation were positive for *Streptococcus hemolyticus*. Cultures from the heart blood, however, gave positive results in about 30% of the animals.

Similar experiment was repeated with SA_{17} strain on hamsters with the same result.

Another experiment was carried out on white mice instead of hamsters with strains SA_{17} and 232. In this experiment, the inter-

[2] Miller, C. P., *Science*, 1933, **78**, 340.

[3] Norton, J. F., and Dingle, J. H., *Am. J. Pub. Health*, 1935, **25**, 609.

TABLE I.
Comparative Lethal Doses of *Streptococcus hemolyticus* for Hamsters.

No. of organism injected									
	SA_{17}	4×10^7	4×10^6	4×10^5	4×10^4	4×10^3	4×10^2	40	4
	E_1	25×10^6	25×10^5	25×10^4	25×10^3	25×10^2	250	25	
Route									
Intraäbdominal	SA_{17}	*1, 1, 1	1, 1, 2	S,S,S	S,S,S	S,S,S	—	—	—
	E_1	1, 1, 1	1, S,S	S,S,S	S,S,S	S,S,S	—	—	—
Interpleural	SA_{17}	—	1, 2, 6	1,17,S	S,S,S	S,S,S	S,S,S	—	—
	E_1	—	1, 1, 1	1, 1, 1	1, S,S	S,S,S	S,S,S	—	—
Intracerebral	SA_{17}	—	—	—	—	1, 1, 1	1, 1, 2	2, 3,S	1, S, S
	E_1	—	—	—	1, 1, 1	1, 1, 1	1, 2, 2	1, 2,S	1, S, S

*Each numeral indicates days of survival of the animal after the infection.
S = Animal remained well 21 days.

TABLE II.
Comparative Lethal Doses of *Streptococcus hemolyticus* for White Mice.

No. of organism injected									
	SA_{17}	3×10^8	3×10^7	3×10^6	3×10^5	3×10^4	1,800	180	18
232	1.P	12×10^7	12×10^6	12×10^5	12×10^4	12×10^3			
	1.C					7,000	700	70	7
	1.V	23×10^6	23×10^5	23×10^4	23×10^3				
Route									
Intraäbdominal	SA_{17}	*1, 1, 1	1, 1, 1	3,S,S	S,S,S	S,S,S	—	—	—
	232	1, 1, 2	2, 2, 6	14,S,S	S,S,S	S,S,S	—	—	—
Intracerebral	SA_{17}	—	—	—	—	—	1, 1, 2	2, 6, 5	1,S,S
	232	—	—	—	—	1, 1, 1	1, 2, 2	2,S,S	1,S,S
Intravenous	232	1, 1, 1	1, 1, 2	3,S,S	S,S,S	—	—	—	—

*Each numeral indicates days of survival of the animal after the infection.
S = Mouse remained well 21 days.

pleural injection was omitted. The amount given intracerebrally was 0.03 cc instead of 0.05 cc. With strain 232, the intravenous route was added and the amount given by this route was 0.1 cc. The result shown in Table II, is just the same as those found in hamsters.

Inoculation by the intracerebral route with strain 232 was repeated 3 months after the first isolation. It was found that the virulence of the organism was markedly decreased.

Studies on the virulence of different strains and different types of *beta Streptococcus hemolyticus* isolated from patients with different diseases and from normal individuals will be reported later.

Conclusion. Hamsters and white mice are suitable animals for testing virulence of *Streptococcus hemolyticus* when injected by the intracerebral route. It requires only 40-400 organisms to kill the animal when given intracerebrally, whereas it requires 400,000 when given interpleurally and 4,000,000-11,500,000 when given intraäbdominally and intravenously.

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Effect of Vitamin C on Creatine and Creatinine Metabolism.

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During a study of creatine and creatinine metabolism in hypothyroidism by one of us,[1] one of the experimental subjects was utilized for studies on vitamin C[2]. It was noted that when the patient was saturated with the vitamin, there was a perceptible increase in the creatine and creatinine excretion. This is shown in Fig. I. Further, the daily fluctuation in the subsequent period seemed to be augmented. We, therefore, repeated the experiment on 4 normal children and on a case of glycogen disease in which the tolerance to creatine has been demonstrated to be much decreased.[3]

Experimental. The subjects were admitted to the metabolic ward

1 Fan, Chuan, Creatine and creatinine metabolism in hypothyroidism, in preparation.

2 Chu, F. T., and Sung, C., *Chinese Med. J.*, 1937, **52**, 791.

3 Fan, Chuan, and Woo, Theresa T., *Chinese Med. J.*, Pediatric Number, 1940.