Enhancement of Pathogenicity of Enterobacteriaceae for Mice by Wetting Agents. (23065)

DACIO DE ALMEIDA CHRISTOVAO (Introduced by G. W. Rake)
Department of Microbiology and Immunology, School of Hygiene and Public Health, University of São Paulo.

The enhancement of pathogenicity of many bacteria under circumstances which have suggested an increase of invasiveness is well known. Typical instances are those of spreading factors discovered by Duran-Reynals(1) and of mucin, as demonstrated by Nungester *et al.*(2), Miller(3,4), and Rake(5,6). While the mode of action of the latter remains controversial(7-11), spreading factors have been identified with hyaluronidase(12,13) and their effect is ascribed to an increase in tissue permeability which results from enzymatic hydrolysis of hyaluronic acid and facilitates penetration and scattering of infectious organisms.

The assumption that wetting agents, which promote closer interfacial contacts, might facilitate access of microorganisms to the intimacy of the host's tissues through liquid or semiliquid media led the author to investigate the effect of surface tension depressants on bacterial infection. In a preliminary investigation(14) the effect of dioctyl sodium sulfosuccinate (Deceresol-OT*) on pathogenicity of *Salmonella typhosa* was determined for mice inoculated intraperitoneally. The innocuousness of this wetting agent having been established for the bacteria and inoculated animals, it was found that Deceresol-OT in 5 tests consistently increased pathogenicity of the organism, as shown by LD_{50}'s 100 to 2,000 times smaller than in the controls. On the basis of this finding, the investigation was extended to include other bacterial species and another wetting agent. The results reported here suggest a possible association between the property of decreasing surface tension and the power to enhance bacterial pathogenicity.

Method. Wetting agents employed were Deceresol-OT and sodium lauryl sulfate (Duponol W.A.). The former is soluble in water at the rate of 1.5 g/l at 20°C and 2.3 g/l at 40°C, soaking of several hours being necessary to dissolve it; the pH of the solution varies from 6.5 to 7.0, and at concentration of 1:1,000 surface tension of water is reduced to 26.8/cm. The latter is a well known and readily soluble wetting agent, used in one of the standard culture media for coliform detection in water.

The effect of Deceresol-OT on pathogenicity of bacteria for mice was tested with *Shigella dysenteriae* and *Shigella paradysenteriae;* that of Duponol W.A. with *Salmonella typhosa* (strain "Panama 58") and *Shigella ambigua*. Suspensions of the organisms in saline were adjusted to known concentrations according to turbidity standards. Starting from one suspension of each organism, further dilutions were prepared with the same ratio, 2, 4 or 5. In any test, 4 to 9 of these dilutions were used in each of 2 series: the controls, containing the organism in saline and the experimental series, to which a solution of wetting agent was added.

Results. Table I gives the final concentrations of wetting agents used for different organisms and the volumes inoculated intraperitoneally into each animal. Five Swiss albino mice weighing 15 to 18 g were inoculated with each dilution of the control or experimental series. In the latter the mice received 1 mg of wetting agent, except those inoculated with *Shigella ambigua,* which received 1.66 mg of Duponol W.A. Preliminary experiments in which 0.5 ml volumes were inoculated intraperitoneally, showed that mice receiving up to 1 mg of Deceresol-OT only occasionally showed some fur ruffling, whereas amounts up to 2.5 mg of Duponol W.A. were well tolerated. As an additional control of the innocuousness of the wetting agents to the experimental animals, in each test an equal amount of the chemical alone in the same total volume of saline was inocu-

* Trademark, Amer. Cyanamid Co.

TABLE I. Effect of Wetting Agents on Pathogenicity of Enterobacteriaceae Inoculated Intraperitoneally into Mice.

Organism	Chemical and conc.	Vol inoculated, ml	LD_{50}* Exp.	LD_{50}* Control
Sh. dysenteriae	Deceresol-OT 1:500	.5	6.72	8.73
Sh. paradysenteriae	" "	"	6.30	8.63
Salm. typhosa	Duponol W.A. "	"	7.52	8.34
Sh. ambigua	" 1:600	1.0	6.81	8.55

* Logarithms to base 10 of No. of organisms killing 50% of inoculated mice in 72 hr, as estimated by the Reed-Muench method from 4 to 9 groups of 5 mice.

lated intraperitoneally into 5 mice: in no case did these mice show any sign of illness.

The median lethal doses were estimated by the Reed-Muench method, and the results, expressed as logarithms to base 10, are presented in Table I. It is clear that there was considerable reduction in the LD_{50} values for the experimental series of each test as compared with the corresponding control, indicating increases of approximately 100, 200, 7 and 60 times in pathogenicity of, respectively, *Sh. dysenteriae, Sh. paradysenteriae, Salm. typhosa,* and *Sh. ambigua.* The effect of Duponol W.A., though quite definite, seems to be less intense than that of Deceresol-OT. Further experiments are necessary to establish whether the enhancement of pathogenicity by wetting agents is peculiar to enteric bacilli inoculated intraperitoneally into mice as well as to elucidate the mechanism of the observed effect.

Summary. Two wetting agents, Deceresol-OT and Duponol W.A., when added to suspensions of *Sh. dysenteriae, Sh. paradysenteriae, Salm. typhosa,* or *Sh. ambigua,* consistently enhanced pathogenicity of the organisms for mice inoculated intraperitoneally.

1. Duran-Reynals, F., *Bact. Rev.,* 1942, v6, 197.
2. Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932-33, v30, 120.
3. Miller, C. P., *Science,* 1933, v78, 340.
4. ———, PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1136, 1138, 1140.
5. Rake, G., *J. Exp. Med.,* 1935, v61, 545.
6. ———, PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1175, 1523.
7. Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Infect. Dis.,* 1936, v59, 11.
8. Anderson, C. G., and Oag, R. K., *Brit. J. Exp. Path.,* 1939, v20, 25.
9. Tunnicliff, R., *J. Infect. Dis.,* 1940, v66, 189.
10. Gould, J. C., and King, H. K., *Biochem. J.,* 1947, v41, xxi.
11. Sandage, C., and Stark, O. K., *J. Infect. Dis.,* 1949, v84, 310.
12. Chain, E., and Duthie, E. S., *Nature, London,* 1939, v133, 977.
13. ———, *Brit. J. Exp. Path.,* 1940, v21, 324.
14. Christovão, D. de A., *Arq. Fac. Hig. Saúde Púb.,* 1955, v9, 149.

Received November 8, 1956. P.S.E.B.M., 1957, v94.

Biochemical and Pharmacological Studies with D- and L-5-Hydroxytryptophan. (23066)

KURT FRETER, HERBERT WEISSBACH, SIDNEY UDENFRIEND AND BERNHARD WITKOP
(Introduced by R. W. Berliner)
Nat. Inst. of Arthritis and Metabolic Diseases, and Nat. Heart Inst., N.I.H., Bethesda, Md.

Following synthesis of 5-hydroxy-D,L-tryptophan (5HTP)(1) it was shown that this amino acid is the precursor of 5-hydroxytryptamine (serotonin)(2) and that this conversion is catalyzed in animal tissues by a specific decarboxylase(3). The amino acid itself has now been found in toad venom(4) in *Chromobacterium violaceum*(5) and in urine of a patient with malignant carcinoid.* It

* C. E. Dalgliesh, personal communication.