may be present in latent form in a considerable number of laboratory mice. The finding that the virus can be activated by the intranasal inoculation of fresh human serum seems of considerable interest.

14433

Effect of Sulfonamides on Toxic and Antigenic Actions of Endotoxins of Certain Gram-Negative Bacteria.

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Four independent groups of workers[1-4] have reported that sulfonamide compounds protect mice to a limited extent against the lethal effects of various bacterial toxins, especially the endotoxins* of gram-negative bacteria. On the other hand, some workers[5-7] failed to observe protective effects in similar experiments.

Hutner and Zahl[3] found that orally administered sulfanilamide protected mice against between one to ten LD_{50} of the endotoxin of *Salmonella typhimurium*. The object of the present study was: (1) to obtain a quantitative evaluation of the relative degree of protection provided by sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, and sulfaguanidine,† (2) to ascertain if this protective action of the sulfonamides affects the immunizing properties of the endotoxins of gram-negative bacteria, as determined by the degree of resistance to the endotoxin developed by mice immunized while receiving sulfonamide treatment.

Relative Protection by Various Sulfonamides. The partially purified endotoxins of *Salmonella typhimurium* and *Shigella paradysenteriæ* Flexner were chosen as representative of endotoxins of gram-negative bacteria generally. These organisms were grown in large quantities in synthetic culture media, using methods described elsewhere.[8] The

[1] Levaditi, C., and Vaisman, A., *C. R. Soc. Biol.*, 1938, **128**, 463; Levaditi, C., Vaisman, A., and Reinié, L., *Ann. Inst. Pasteur*, 1938, **61**, 635.

[2] Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530; Carpenter, C. M., and Barbour, G. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 255; Carpenter, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 354; Carpenter, C. M., *Rep. Proc. 3rd Internat. Congress Microbiology*, 1940, 595.

[3] Hutner, S. H., and Zahl, Paul A., *Science*, 1942, **96**, 563; Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285.

[4] Birger, O. G., and Hal'perin, E. P., *Zeit. Mikrobiol., Epidemiol., Immun.* (U.S.S.R.), 1941, **5-6**, 94.

* For most gram-negative bacteria, the toxic factor present in the bodies of the organisms is probably part of the toxic principle somatic O antigen. In *Neisseria*, some species of *Hemophilus* and *Brucella*, and possibly the rough phase of most gram-negative bacteria, the toxin may be part of a nucleoprotein, different in chemical pattern from the O antigens studied by Boivin, Morgan and Partridge, and others. However, the mode of pathogenesis of both toxins appears similar, if not identical. We have therefore for these studies preferred the more inclusive term "endotoxin" or "toxic factor" rather than the more precise immunochemical descriptions.

[5] Long, P. H., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine, and Allied Compounds*, 1939, Macmillan Company, New York City.

[6] Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

[7] Gross, P., Cooper, F. B., and Lewis, M., *J. Inf. Dis.*, 1938, **63**, 245.

† We wish to express our appreciation to Lederle Laboratories, Inc., and to the American Cyanamide Company for generous gifts of sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazine, and to Sharp and Dohme for sulfamerazine.

[8] Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, in press.

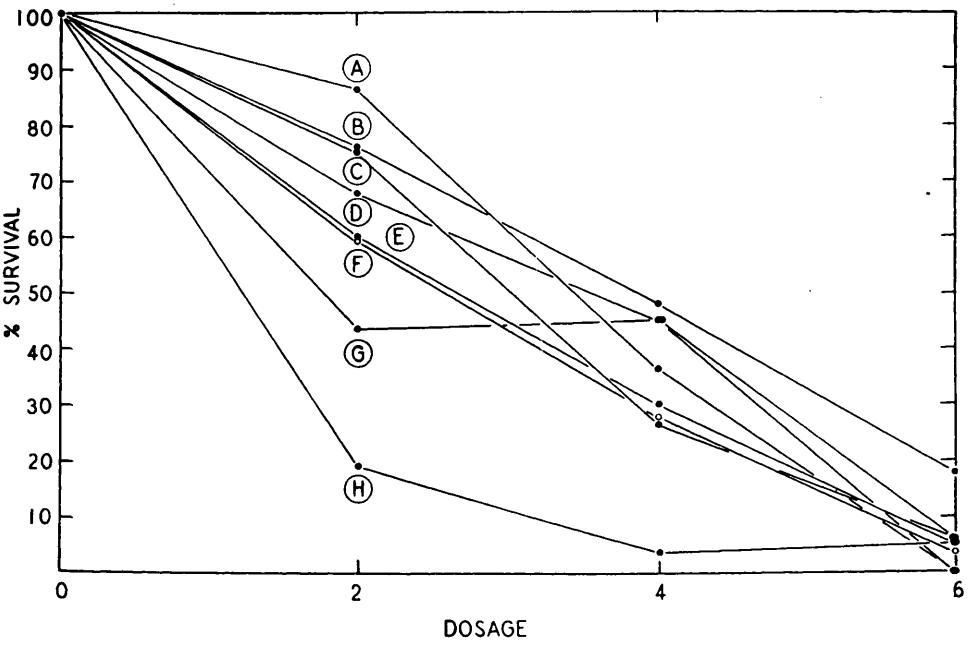

FIG. 1.

Effect of orally administered sulfonamides on the survival of mice receiving injections of *Salmonella* and *Shigella* endotoxins. Dosage is LD_{50} defined as amount of endotoxin required to kill within 24 hours 50% of mice injected intraperitoneally. In dry weight the amount of toxic material constituting 1 LD_{50} for *Salmonella* is 2.2 mg; for *Shigella*, 8.0 mg.

A—sulfanilamide; B—sulfaguanidine; C—sulfathiazole; D—sulfadiazine; E—sulfamerazine; F—sulfanilamide (*Shigella*); G—sulfapyridine; H—controls. The data of curve (F) is for mice receiving sulfanilamide following the injection of *Shigella* endotoxin. The remainder of the data applies to *Salmonella*.

Each plotted point represents 16 to 38 animals. The control points represent 50 to 76 animals. The total data plotted represent 659 mice.

cultures were killed by adding phenol to 2%, reduced in volume by pervaporation, precipitated from 66% acetone, and dried with acetone and ether. Aqueous dispersions of the precipitates, injected intraperitoneally, were used for all tests.

Control data on the survival of mice after the injection of the endotoxins at 3 dosage levels were obtained by observing the number of mice surviving at the end of 24 hours. The dosage levels chosen were 2, 4, and 6 LD_{50}. The LD_{50} (see caption to Fig. 1) is based on dose-survival curves published elsewhere.[8] The protective effects of the sulfonamides on the endotoxin-treated mice was determined by administering the sulfa drugs by means of a stomach tube attached to a tuberculin syringe, and observing the number of mice surviving at the end of 24 hours. In view of the relative absence of toxic effects from large doses of the sulfonamides in mice, an arbitrary dose of 25 mg of the sulfonamides in aqueous suspension was selected. The sulfonamides were administered a few minutes before the intraperitoneal injection of the endotoxin. The number of mice surviving for 24 hours provided the data plotted in Fig. 1.

Immunization During Sulfonamide Treatment. To determine whether the sulfonamides interfere with the immunizing properties of the *Salmonella* endotoxin while exerting protection against the toxic material, the following experiments were performed. In Experiment 1 the mice of Group A (50 male Rockland mice of 18-20 g) were given injections of the endotoxin in increasing doses according to the schedule listed in Table I. The number of animals surviving each injection was recorded. Several days after the last injection on the immunizing schedule, a very heavy dose (10 LD_{50}—lethal to all the controls) was injected. The survival data follow-

TABLE I.

Survival of Mice Receiving Sulfathiazole Treatment While Being Immunized with the Endotoxin of *Salmonella typhimurium*. Exp. No. 1, Group A, is control (without sulfathiazole) series; Exp. No. 1, Group B, is series receiving sulfathiazole during immunization. Exp. No. 2 is similar to No. 1, except that the dosages for immunization are higher and the schedule is somewhat altered. For definition of LD_{50} see caption to Fig. 1.

	Group A			Group B		
Days after 1st inj.	Dose of *Salmonella* endotoxin, LD_{50}	Dose of sulfathiazole (oral)	No. of mice surviving preceding inj.	Dose of *Salmonella* endotoxin, LD_{50}	Dose of sulfathiazole (oral) mg	No. of mice surviving preceding inj.
			Exp. No. 1.			
0	1/8	—	50	1/8	25	50
2	1/2	—	48	1/2	25	50
5	1	—	40	1	25	48
7	1	—	38	1	25	46
9	2	—	35	2	25	44
13	4	—	32	4	—	44
17	10	—	28	10	—	38
18	—	—	11	—	—	16
			Exp. No. 2.			
	Group C			Group D		
0	1	—	50	1	25	50
2	2	—	31	2	25	44
5	4	—	24	4	25	40
7	4	—	16	4	25	34
12	10	—	14	10	—	32
13	—	—	5	—	—	9

ing this massive injection was taken as an index of the degree of immunity which had developed in these control animals. Concurrently another series of 50 animals (Group B) was set up in which the mice, in addition to receiving the immunizing injections of endotoxin, were given 25 mg oral doses of sulfathiazole. This dose of sulfathiazole accompanied each immunizing injection, but was *not* given with the final massive assay injection.

In Experiment 2 the above steps were repeated but at higher dosage levels of immunizing injections. The data for the control (Group C) and for the sulfathiazole-treated animals (Group D) are also listed in Table I.

Results and Discussion. One may conclude from Fig. 1 that the 6 sulfonamides tested confer definite but limited protective effects of comparable magnitude against the lethal action of the *Salmonella* endotoxin, and that sulfanilamide at least has a similar effect on the *Shigella* endotoxin.

The failure of several investigators[5-7] to observe a protective effect of the sulfonamides on bacterial endotoxins is difficult to explain, except possibly on the basis that the effect, being small, might have been overlooked unless large numbers of animals were used and doses carefully controlled. It is apparent from our experience that if the assay dose had been in excess of 4-6 LD_{50}, the protective effect of the sulfonamides would probably have been missed. In addition much of the data cited by these workers concerns toxins from gram-positive organisms. The question of protection against toxins of gram-positive organisms falls outside the scope of this paper.

It appears probable that the protective action of the sulfonamides against the toxic preparation used in our experiments is largely referable to the O antigen component, and not to unidentified impurities. This conclusion is based: (1) on the demonstration by Boivin and Mesrobeanu[9] and other workers that the principal toxic component of the cells of gram-negative bacteria in the smooth phase is found in the O antigen, (2) on the reports of Levaditi, Vaisman, and Reinié[1] of a protective effect of sulfonamides against highly purified O antigens of gonococcus, meningococcus, *Salmonella, Shigella* and *Pasteurella*

[9] Boivin, A., and Mesrobeanu, L., *Ann. Inst. Pasteur*, 1938, **61**, 426.

prepared according to Boivin's trichloracetic acid technic.

The protective effect of sulfonamides appears to admit of at least 2 possible explanations: (1) that the action is directly on the toxic O antigen, *i.e.*, the sulfonamides participate in a detoxication process, (2) that the action is one of enhancing the general resistance of the body to the toxic material. The possibility of a direct effect of sulfonamide on the endotoxin appears to be ruled out by the reports of both the Levaditi and Carpenter groups that the toxicity of the endotoxin was not permanently reduced after incubation *in vitro* with sulfonamides.

While this conclusion appears to oppose the hypothesis that a detoxication process is involved in the effect of sulfonamides on the action of gram-negative endotoxins, the hypothesis cannot on this basis be dismissed entirely; for even if a major portion of the antigen were removed by sulfonamide detoxication, enough could conceivably have remained to elicit the observed antibody response. It must be borne in mind that we did not determine the minimal amounts of antigen required for immunization.

An examination of Table I indicates that a comparable degree of immunity was developed in mice subjected to a particular course of immunization, irrespective of whether sulfathiazole was administered concurrently (Groups B and D) or was not administered (Groups A and C). Mason[10] reported that rabbits undergoing immunization with pneumococci while being treated with sulfonamides developed the same antibody titer as rabbits not receiving sulfonamide treatment. Finland, Spring, and Lowell[11] obtained similar results in human pneumococcus pneumonia when sulfapyridine was used. We conclude from these observations that the sulfonamides did not influence the antigen-antibody reaction in respect to the gram-positive pneumococci. Our experiments indicate a similar lack of interference with immunization against the endotoxins of gram-negative organisms.

Our previous work[12,13] indicates that a similar mode of action characterizes the endotoxins of almost all gram-negative bacteria; and it is generally considered that the sulfa drugs, while varying in clinical efficacy, are also similar in mode of action. Although the experimental work reported here was confined to a test of *Salmonella* and *Shigella* endotoxins, and to a limited number of sulfa drugs, it is reasonable to expect that similar results would have been obtained with other sulfa drugs and with the endotoxins of other gram-negative bacteria.

In view of the protective action of sulfonamides against endotoxins of gram-negative organisms and the absence of gross interference with the immunization process, at least in so far as immunity to the endotoxin is concerned, there is a possibility that sulfa drugs may be of clinical value in mitigating certain undesirable symptoms which often accompany the use of typhoid, dysentery, and other vaccines prepared with gram-negative organisms.

Summary. Orally administered sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, and sulfaguanidine confer an equivalent but limited protective effect against the lethal action of intraperitoneally-administered endotoxin of *Salmonella typhimurium*. Sulfanilamide has a similar effect on the endotoxin of *Shigella paradysenteriæ* Flexner. Sulfathiazole administered during the immunization of mice with *Salmonella* endotoxin appears not to interfere with the immunizing process, as determined by degree of resistance to the lethal action of the endotoxin. Reasons are advanced to support the view that these results apply to other sulfonamide drugs and to the endotoxins of gram-negative bacteria generally.

[10] Mason, M. M., *Vet. Med.*, 1942, **37**, 30.

[11] Finland, M., Spring, W. C., and Lowell, F. L., *J. Clin. Invest.*, 1940, **19**, 179.

[12] Zahl, Paul A., Hutner, S. H., Spitz, S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.

[13] Hutner, S. H., and Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 364.