ficient animals was 344 per min, compared to 445 per min for controls. This degree of bradycardia was not reflected, however, in the beat of the isolated auricles, the mean rates for the two groups being 216 per min and 234 per min respectively. Addition of thiamine chloride to isolated auricles caused no greater increase of rate in those from deficient animals than in controls. MacDonald and McHenry[3] have shown that the bradycardia in intact animals is not relieved by administration of thiamine unless food is also given.

The bradycardia would not seem to be a function of metabolic changes in the heart muscle as, for example, the tachycardia of hyperthyroidism, which persists after the auricles are isolated from the intact animal.[4] It is possible that chemical factors responsible for bradycardia might diffuse out of the myocardium during the period of isolation but it is more likely that the bradycardia is of neurogenic origin.

Summary. The bradycardia of thiamine-deficient rats disappears when the auricles are isolated *in vitro*. It is probably of neurogenic origin.

[3] MacDonald, D. G. H., and McHenry, E. W., *Am. J. Physiol.*, 1940, **128**, 608.

[4] Lewis, J. K., and McEachern, D., *Bull. Johns Hopkins Hosp.*, 1931, **48**, 228.

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Protection of Mice Against Meningococcal Infection by Sulfadiazine, and Inhibition of Protection by Para-aminobenzoic Acid.

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During the course of an investigation into the immunological response of patients following meningococcal infection,[1] a number of mouse-protection tests were undertaken with serums obtained from patients during the early and convalescent stages of the disease. All of these patients with meningitis or meningococcemia had received treatment with sulfonamides, and most of them received sulfadiazine. It was noted that mice were protected against multiple lethal doses of meningococci by serum containing sulfadiazine, even in amounts less than 1 mg %, regardless of the stage of illness during which the serum was obtained from the patient. The addition of para-aminobenzoic acid to such serum, in a concentration of 5 mg %, had no inhibitory effect on this protection. Similar protective action was exerted by small amounts of sulfadiazine diluted in normal serum or in physiological saline, and again the addition of para-aminobenzoic acid was without effect. Because of these observations, further investigations were carried out in order to determine (1) the amount of sulfadiazine necessary to induce protection of mice against a virulent strain of Group I meningococcus and (2) under what circumstances this protection was inhibited by para-aminobenzoic acid.

Materials and Methods. Three solutions of sodium sulfadiazine in physiological saline were made, containing 1.0, 0.5 and 0.1 mg per 100 ml. Similar concentrations of the drug were prepared in normal human serum which had been diluted 1:5 in saline. These were injected intraperitoneally into 3 groups of mice, in 0.5 ml amounts, so that the doses for each group were 0.005, 0.0025 and 0.0005 mg, respectively. These injections were made 30 min before the injection of organisms.

The following methods were employed for

* Fellow of the Frederick Tilney Memorial Fund.

[1] Thomas, L., Smith, H. W., and Dingle, J. H., submitted for publication.

TABLE I.
Protective Action of Sulfadiazine for Mice Inoculated Intraperitoneally with Group I Meningococci, and Effect of Para-aminobenzoic Acid upon This Protection.

Sulfadiazine mg	Para-aminobenzoic acid	No. of meningococci injected* 100,000	10,000	1,000	100
.005	0	0†	0	0	0
.0025		0	0	0	0
.0005		2	2	2	0
.005	0.1 mg intraperitoneally with sulfadiazine	0	0	0	0
.0025		0	0	0	0
.0005		2	2	1	1
.0025	0.1 mg intraperitoneally with sulfadiazine, followed by 0.5 mg subcutaneously with organisms	0	0	0	0
.005	0.1 mg intraperitoneally with sulfadiazine, and 0.5 mg subcutaneously with organ-	2	2	2	2
.0005	isms, followed by 0.5 mg subcutaneously every 3 hrs for 21 hrs	2	2	2	2
0	0	2	2	2	2

*1.0 ml of a 3% suspension of mucin containing the given number of meningococci was inoculated intraperitoneally 30 minutes after the administration of sulfadiazine.

†Two mice were inoculated in each group. Each numeral represents the number of mice dying.

the administration of para-aminobenzoic acid: (1) 0.1 mg of para-aminobenzoic acid was added to the sulfadiazine before the intraperitoneal injection of the latter. (2) 0.5 mg of para-aminobenzoic acid, dissolved in 0.5 ml of distilled water, was injected subcutaneously into the abdominal wall at the time of the inoculation of organisms, *i.e.*, 30 min after the injection of sulfadiazine. (3) Eight subcutaneous injections of 0.5 mg of para-aminobenzoic acid were made at 3-hr intervals, beginning at the time of the injection of organisms.

Swiss mice, weighing between 15 and 18 g, were used. All were from a single breed. A 3% suspension of hog mucin in distilled water was prepared by mixing in a Waring Blendor, and was then sterilized by autoclaving and adjusted to pH 7.4.

The same strain of Group I meningococcus (strain No. 2) was used in all the tests. This strain was isolated from the spinal fluid of a patient, and had been maintained in the frozen state. Previous titrations of the virulence for mice had shown the 50% lethal dose to be ten organisms. Titration of the *in vitro* action of sulfadiazine against this strain in a synthetic medium[2] had shown that 0.04 mg % of the drug was bacteriostatic, and 0.1 mg % was bactericidal in a culture originally containing 430 organisms per ml.†

Dilutions of the organisms in mucin were made so that the 10^{-7} dilution contained approximately 100 organisms per cm^3. The dilutions employed for the injection of mice were 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. In each experiment, 2 mice were injected intraperitoneally with 1 ml of each dilution of organisms.

The mice were observed for 72 hr after the injection of organisms, although almost all deaths occurred within the first 24 hr. Cultures of the heart's blood were made on all mice shortly after death, and these were positive for meningococci in all instances.

Results. The results were clear-cut and confirmed the previous observations made with the serum from patients. Complete protection against 100,000 organisms, or 10,000 50% lethal doses, was afforded by 0.0025 mg of sulfadiazine, as is shown by the experiment given in Table I. Protection against 100 or-

[2] Frantz, I., *J. Bact.*, 1942.

† These experiments were carried out by Mrs. Laura J. Ingraham.

ganisms occurred with 0.0005 mg of sulfadiazine. In 2 other groups of experiments, the same degree of protection by sulfadiazine occurred when the drug was dissolved either in physiological saline or in normal human serum, diluted 1:5. The administration of 0.1 mg of para-aminobenzoic acid with the sulfadiazine did not alter the protection, nor did the additional injection of 0.5 mg 30 min later, at the time of the injection of organisms. When repeated subcutaneous injections of para-aminobenzoic acid were made in 0.5 mg amounts, at 3-hr intervals, however, all of the mice died within 21 hr. Normal control mice injected with the same doses of organisms, but without sulfadiazine or para-aminobenzoic acid, all died within 21 hr. Twelve normal mice, not included in the table, were injected subcutaneously at 3-hr intervals with 8 doses of para-aminobenzoic acid, each of 0.5 mg, but no sulfadiazine was given. All of these mice survived.

Comment. The failure of a single administration of para-aminobenzoic acid to inhibit the protective effect of sulfadiazine may be due to two factors: (1) The rapid excretion of para-aminobenzoic acid from the body in contrast to the relatively slow excretion of sulfadiazine. Experimental evidence of this difference has been reported for man[3,4] and for the rabbit,[5] although comparable studies have not yet been carried out in mice. (2) The extreme sensitivity of the meningococcus to small amounts of sulfadiazine. This is indicated by the *in vitro* tests and also by the fact that mice are fully protected against this particular strain of Group I meningococcus by as little as 0.0025 mg of drug.

The successful inhibition of the action of sulfadiazine by repeated subcutaneous injections of para-aminobenzoic acid may best be explained by assuming that a considerable concentration of the latter drug is maintained for a prolonged period, thus producing a constant inhibitory effect. A primary toxic action of the para-aminobenzoic acid itself is, of course, a factor which must also be considered, although control mice survived parallel injections of para-aminobenzoic acid and the total dose of para-aminobenzoic acid was less than 6.0% of the amount which Scott and Robbins[6] have reported as lethal for mice in a single intravenous injection.

Dr. George F. Badger has kindly tested the statistical significance of the above data and has come to the following conclusion: "Due to the consistent results obtained with the various drug dosages, these results are statistically significant."

The results of these experiments indicate that the mouse protection test is an impractical and unreliable method for assessing the antibody titers of sera from patients who have recently received sulfonamide therapy.

Summary. (1) 0.0025 mg of sulfadiazine is sufficient to protect mice against from 10 to 10,000 50% lethal doses of a virulent strain of Group I meningococcus. (2) The protective action of sulfadiazine is not inhibited by a single administration of para-aminobenzoic acid accompanying the sulfadiazine, nor by a second injection after 30 min. (3) The protective action of sulfadiazine may be inhibited by repeated subcutaneous injections of para-aminobenzoic acid at 3-hr intervals.

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[3] Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1941, **20**, 189.

[4] Peterson, O. L., Strauss, E., Taylor, F. H. L., and Finland, M., *Am. J. M. Sc.*, 1941, **201**, 357.

[5] Harrow, B., Mazur, A., and Sherwin, C. P., *J. Biol. Chem.*, 1933, **102**, 35.

[6] Scott, C. C., and Robbins, E. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 184.