stances(4). Further information concerning degradation of mucopolysaccharide might also be gained by study of the sponge tissue in view of the decreasing mucopolysaccharide content of this tissue after the second week.

Summary. Polyvinyl sponges inserted under the dorsal skin of guinea pigs fill with cellular connective tissue. Dry weight of the granulation tissue, concentration of protein, desoxyribosenucleic acid and acid mucopolysaccharide at intervals between 1 and 12 weeks are reported. Both glucosamine and galactosamine were present in the mucopolysaccharide fraction. Evidence for the presence of 3 types of mucopolysaccharide components was obtained by chromatography and carbazole/orcinol ratios. A decrease in carbazole/orcinol ratios suggested an increased proportion of chondroitin sulfate B in the older granulomas.

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Chemotherapeutic Studies with 2,4-Dimethoxy-6-sulfanilamido-1,3-diazine (Madribon*) (24370)

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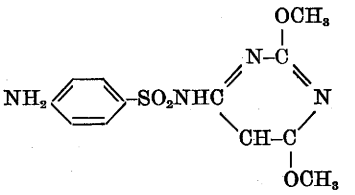
Comparatively high activity of 2,4-dimethoxy-6-sulfanilamido-1,3-diazine in experimental streptococcal infections has been described by Semenitz(1). The results appeared promising although the experiments were carried out with organisms of low virulence and evaluation of the effect of subcutaneous administration was based on very short observation time. More extensive studies on chemotherapeutic activity carried out recently in these laboratories confirmed the remarkable activity of this compound in a series of experimental infections with gram-positive and gram-negative bacteria. The experiments are here described.

Chemical and physical properties. 2,4-Dimethoxy-6-sulfanilamido-1,3-diazine (I; Madribon), first synthesized by Bretschneider and Kloetzer(2), is a white, odorless and almost tasteless crystalline powder with a melting range of 200-202°C. Two Kpa values at 2 and 6 were observed.† The substance is slightly soluble in water, dependent on H-ion concentration. Solubility values determined at 37°C of 4.6 to 7.5 mg/100 ml at pH 4.10 and 5.7 rose to 29.5 mg/100 ml at pH 6.7, 58.0 mg/100 ml at 7.06, 920 mg/100 ml at 8.19 and reached 5170 mg/100 ml at pH 8.71. Under the same conditions, the N^4-acetyl de-



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† We are greatly obliged to Dr. A. Motchane of the Physico-chemical Lab. for permission to include these values as well as data on solubility, in this paper.

rivative was of somewhat lower solubility except in the alkaline range of pH 8.83. These solubility values are not of practical significance because the drug is eliminated in the form of its highly soluble glucuronide (Dr. B. Koechlin; personal communication).

Materials and methods. Antibacterial activity. (a) *in vitro.* The bacteriostatic effect was determined by conventional technic of serial dilution test in the semi-synthetic medium of Adams and Roe(3). The inoculum consisted of 0.05 ml of a 10^{-3} diluted overnight broth culture, but in case of streptococci and pneumococci the same volume of a 10^{-1} diluted culture was used. The drug was dissolved in alkali to give a solution of pH 9.0. The results were read after 24-48 hours' incubation and verified by subculture on appropriate solid media if necessary. (b) *in vivo.* All experiments were carried out in white mice of 18 to 20 g body weight. The procedure followed closely the technic described previously(4,5) and consisted of intra-abdominal infection with 0.5 ml containing 100-1000 minimal lethal doses, using either a properly diluted overnight broth culture of streptococci, pneumococci, Listeria, Klebsiella, or diluted suspensions in 5% gastric mucin in experiments with staphylococci, meningococci, members of the coli-salmonella group, Pasteurella, *P. vulgaris* and *Ps. aeruginosa.* The infective doses used are given in Tables I and II. Graded doses of drug suspended in 5% gum arabic or in oil in water lipid suspension (5) were given by gavage. The dosage schedule consisted either of a single dose given shortly after the infection or in a series of 4 to 6 treatments distributed over 4 days with the first dose also administered shortly after infection. Groups of 10 mice served for every dose. Mice which died were autopsied and cultures taken of blood. Survivors were observed for 3 weeks. Untreated controls infected with the same and lower doses were included in every experiment.

Results. Toxicity. Studies on acute and chronic toxicity were carried out in different species of animals by Randall and his associates(6). It might suffice to mention here that mice tolerated single oral doses of 10,000 mg/kg without signs of toxicity and that administration of 200 mg/kg/day to rats for 13 weeks did not produce pathological changes in tissues although a certain growth inhibition was observed. Determination of the fate of Madribon indicated that it belongs to the group of sulfonamides with prolonged persistence in the body characterized by a half-life of 48 hours in blood of rats.

Antibacterial activity in vitro. Determination of bacteriostatic activity *in vitro* showed the familiar picture of sensitivity of the different organisms characteristic for many sulfonamides. In the group of gram-positive organisms only staphylococci showed high sensitivity with minimal growth inhibiting concentrations of 1.9 μg/ml (strain 209) and 15.6 μg/ml (strain Smith), whereas β-hemolytic streptococci (strain #4), type I pneumococci (ATCC 6301) and *L. monocytogenes* were inhibited by high concentrations of 2000, 500 and 1000 μg/ml respectively. Gram-negative organisms exhibited as a rule higher sensitivity. The values were for *E. coli* (J) 1.9 μg/ml, *K. pneumoniae* 3.9 μg/ml, *S. typhosa* (2 strains) 3.9 μg/ml, *S. typhimurium* 62.5 μg/ml and *S. schottmuelleri* 125 μg/ml. Growth inhibition of *P. multocida* and *Ps. aeruginosa* was not observed with concentrations as high as 2000.0 μg/ml.

Antibacterial activity in vivo. Results of experiments in which the protective activity of Madribon was determined in infections with 12 different organisms are given in Table I. From the figures, which are based on frequently repeated experiments, it may be seen that the compound was effective in the majority of infections tested at doses generally well below 100 mg/kg even if only a single treatment was given, *e.g.* in Salmonella infections. The values are comparable to those observed with highly active sulfonamides, *e.g.* sulfadiazine and particularly sulfamethoxypyridazine, as determined in our laboratory and elsewhere (7). In case of the pneumococcal and Pseudomonas infections which are known to respond less readily to sulfonamides, higher but still comparatively low doses were required and the values were more favorable than could be expected from the bacteriostatic effect *in vitro.* It is also noteworthy that hemolytic streptococci and *P. multocida* responded to

TABLE I. *In Vivo* Antibacterial Activity of Madribon.

Organism	Infective dose: 0.5 ml	No. of mice	No. of doses	PD_{50}, mg/kg*	% mortality of controls
S. pyogenes #4	10^{-5}	420	6	43.2	98.3
D. pneumoniae #6301	10^{-6}	210	6	423.0	100.0
M. pyogenes var. *aureus* (Smith)	10^{-5}	180	4	52.1	93.3
L. monocytogenes	10^{-1}	250	6	43.8	88.0
N. meningitidis	"	120	1	44.4	95.0
P. multocida	10^{-6}	90	6	24.5	85.0
E. coli J	10^{-8}	120	4	88.9	95.0
K. pneumoniae	"	320	6	71.7	100.0
S. schottmuelleri	10^{-6}	340	1	53.5	94.0
S. typhosa P58a	10^{-4}	190	1	10.8	96.7
Ps. aeruginosa	"	240	4	325.0	82.5
P. vulgaris	10^{-6}	130	1	34.9	80.0

* 50% protective dose, calculated according to Reed and Muench(8).

low doses *in vivo* despite their lack of sensitivity *in vitro*.

The streptococcal, staphylococcal and Listeria infections were of approximately equal susceptibility in the group of gram-positive organisms. Among gram-negative bacteria *S. typhosa* showed the highest sensitivity which is characteristic for many sulfonamides. It might, however, be mentioned that Madribon had no effect on oral infection of mice with *S. typhimurium*. A diet containing 1% or 2% of the drug corresponding to 2.5 to 5.0 g/kg/day given for 7 days, failed to protect a significant number of mice against the fatal course of the disease. Death occurred after an average survival time of 15-16 days in this as well as in the control group. Not given in the Table are experiments with entero-pathogenic strains of *E. coli*, of the serotypes O 119, O 125, O 126 and O 128. Although intra-abdominal infections of more than 1000 minimal lethal doses were used and only a single oral dose was given, an average 50% protective dose of 32.0 mg/kg (min. 25.7, max. 36.1) was obtained.

TABLE II. Antibacterial *In Vivo* Activity of Madribon under Conditions of Single Drug Administration in a Lipid (Oil in Water) Emulsion.

Organism	Infective dose: 0.5 ml	PD_{50}, mg/kg*	% mortality of controls
S. pyogenes #4	10^{-6}	103.3	100
D. pneumoniae #6301	"	732.1	100
E. coli J	10^{-8}	80.7	90
S. typhosa P58a	10^{-5}	17.5	100
S. schottmuelleri	10^{-6}	33.3	90
P. vulgaris	10^{-5}	42.6	80
Ps. aeruginosa	"	424.0	70

* 50% protective dose, according to Reed and Muench(8).

In an additional series of experiments compiled in Table II the effect of single dose treatment was determined in 7 different infections. Suspension of the drug in an oil in water emulsion was selected. It is evident that Madribon exerted its protective activity in all instances. The influence of the lipid vehicle as compared to gum arabic was negligible in infections with gram-negative organisms (Table I) and also in the streptococcal infection, which responded to a single dose of Madribon in gum with a PD_{50} of 178 mg/kg. Enhancement by suspension in oil in water emulsion was only noticeable in pneumococci which otherwise do not respond to single treatment with sulfonamides.

Discussion. The antibacterial *in vivo* activity of Madribon is characterized by its scope and its intensity. All 12 organisms tested, 4 gram-positive and 8 gram-negative ones, responded to comparatively small doses of the drug. With a few exceptions (*D. pneumoniae, Ps. aeruginosa*) the 50% protective doses were less than 100 mg/kg with an average of approximately 46 mg/kg for gram-positive and 49 mg/kg for gram-negative organisms. This type of marked activity must be ascribed to the high chemotherapeutic potency exerted *in vivo* though not in all instances reflected by the bacteriostatic effect *in vitro*. Another aspect of the Madribon activity, namely reduction of frequency of treatment to a single dose even in streptococcal and pneumococcal infections, can probably be

attributed to the persistence of effective concentrations in blood and tissues.

Whether the presence of the methoxy groups is correlated to toxicological and chemotherapeutic characteristics of Madribon cannot yet be decided, although the influence of the methoxy group on the properties of sulfamethoxypyrimidine might allow such assumption by analogy.

Summary. A new sulfonamide, Madribon, exerted high protective activity in 12 experimental bacterial infections produced by gram-positive and gram-negative organisms. The frequency of drug administration could be reduced to a single dose in all 7 infections in which this technic was tried.

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Dietary Saponin and Plasma Cholesterol in the Chicken.*† (24371)

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Considerable interest exists in factors influencing plasma cholesterol level. Hypercholesterolemia induced by cholesterol feeding is now a widely known phenomenon in the chicken. Recently, Newman and co-workers (1) have demonstrated a decrease in serum cholesterol (and also liver cholesterol) of chicks which were fed cholesterol and saponin simultaneously. Schoenheimer and Sperry (2) have shown that saponins have the property of forming stable complexes with cholesterol; therefore, a reduction of blood cholesterol due to feeding of saponin with a cholesterol containing diet might be expected. Blood cholesterol level of chicks however, can be significantly varied not only by dietary cholesterol, but also by other factors such as exercise(3), and a low level of protein in the diet (4,5). In the present report an attempt was made to evaluate the influence of saponin on endogenous hypercholesterolemia in chickens, induced by feeding low protein diets.

Methods. Three experiments were performed. In Exp. 1, male crossbred chicks were started on experimental diets at one day of age, and at 24 days blood was taken for plasma cholesterol analysis. In Exp. 2, newly hatched female cross-bred chicks were first kept on conventional starter mash for 1 week, and plasma analyzed for cholesterol after 16 and 23 days on the experimental diets. In Exp. 3, 1-year-old White Leghorn roosters were used, and their plasma cholesterol determined 10 and 20 days after the birds had been put on experimental diets. Composition of diets is given in Table I, and dietary additives to the different lots appear in Table II. Ten chicks or 5 adult birds were kept on each treatment in each experiment. Chicks were housed in electrically heated and thermo-

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