16469

Chemotherapy of Joint Involvement in Mice Produced by *Streptobacillus moniliformis.*

JENNIE S. LEVEY AND STANLEY LEVEY. (Introduced by Arthur H. Smith.)

From the Wayne County General Hospital, Eloise, and the Department of Physiological Chemistry, Wayne University College of Medicine, Detroit.

Both in rat bite fever and in Haverhill fever where *Streptobacillus moniliformis* is the etiological agent. patients may experience joint involvement among other symptoms.[1-4] This microorganism has also been recovered from mice which have developed spontaneous arthritis.[1,5] Most of the chemotherapeutic studies involving *Streptobacillus moniliformis* have been concerned with the protective effect of the various agents when administered prior to or at the time of inoculation of the test animals. In the present study the animals were inoculated and allowed to develop swollen joints before the test agents were administered. In this manner it was possible to evaluate the therapeutic effect of various substances. The drugs tested were chosen because they possess chemotherapeutic value in various other infectious diseases and in addition possess different chemical structures.

Methods. The Manning strain of *Streptobacillus moniliformis* used in this work was supplied by Dr. Carl E. Duffy, Wayne University College of Medicine. It was isolated from the blood of a man who had developed painful and swollen joints following a fall. There was no history of a rat bite. The typical "fluff-ball" growth of this microorganism appeared readily in either tryptose phosphate or freshly prepared beef heart infusion broth[6] supplemented with Seitz-filtered bovine serum to a final concentration of 20%.

Broth cultures of low passages of the Manning strain were stored in small amounts in solid carbon dioxide. When needed, they were thawed quickly in a water bath of 37°C and transferred to flasks of sterile 20% bovine serum broth. Two to five-tenths of a ml of either a 24- or 48-hour broth culture was administered intravenously to mice weighing approximately 20 g. Subcultures up to the seventh passage were used. The microorganism proved to be so virulent for these mice that extremely swollen joints appeared as early as 2 days after the inoculation. If the individual subculture caused the death of most of the animals 48 hours after inoculation, the smaller dose level (0.2 ml) was used. Any of the mice which failed to show swollen joints were discarded.

The substances tested were: penicillin G, streptomycin, Myochrysine (gold sodium thiomalate), Solgonal B* (gold thioglucose in oil), sulfathiazole, p-aminosalicylic acid,† p-aminobenzoic acid, promizole† (2-amino-5-sulfanilylthiazole), promin† (disodium salt of P, P′ diamino-diphenylsulfone-N, N′ diglucose sulfonate) and methylene blue. Therapy was begun 3 days after the appearance of one or more swollen joints. Since the mode of treatment varied with the individual drug, the dosage will be described in the discussion of the specific agent. The evaluation of the therapy depended on the complete disappearance grossly of all joint involvement. Reduc-

1 Brown, T. McP., and Nunemaker, J. C., *Bull. Johns Hopkins Hosp.*, 1942, **70**, 201.

2 Place, E. H., Sutton, L. E., and Willner, O., *Boston M. and S. J.*, 1926, **194**, 285.

3 Place, E. H., and Sutton, L. E., *Arch. Int. Med.*, 1934, **54**, 659.

4 Parker, F., Jr., and Hudson, N. P., *Am. J. Path.*, 1926, **2**, 357.

5 Williams, S., *M. J. Australia*, 1941, **1**, 357.

6 Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology*, D. Appleton-Century Co., New York, 1935, p. 1056.

* This compound was supplied through the courtesy of the Schering Laboratory, Bloomingdale, N.J.

† These compounds were supplied through the courtesy of Dr. L. L. Bambas of Parke, Davis and Company, Detroit, Mich.

TABLE I.
Effect of Various Agents upon Joint Involvement Produced by *Streptobacillus moniliformis.*

Group	Drug	Course of therapy	No. of animals tested	No. of animals cured	% cured
1	Control		63	1	1.6
2	Penicillin G	10,000 units I.P., 3 × daily, 4 days	32	12	37.5
		1,000 " " " " " 6 "	16	11	69.0
3	Streptomycin	5,000 " " " " " 3 "	7	6	85.0
		2,500 " " " " " 3 "	17	13	76.0
4	Myochrysine	1.25 mg I.V. every other day, 10 doses	17	0	0
		5 mg I.P. single dose	5	0	0
		4.0 mg I.V. every other day, 10 doses	8	0	0
5	Solgonal-B in oil	10 mg I.M. single dose	7	0	0
6	Sulfathiazole	1 % in diet, 38 days	11	0	0
7	*p*-Aminosalicylic acid	.5% " " 9 "	13	0	0
8	*p*-Aminobenzoic acid	.4% " " 21 "	10	0	0
9	Promizole	.5% " " 5 "	13	1 (?)	0
10	Methylene blue	.2% " " 20 "	5	0	0
11	Promin	.5% " " 21 "	22	0	0

tion of the number of limbs involved without complete remission was not considered a cure. The mice were observed for a period of 3 months; if any of the mice died during the first 3 days of treatment their records were discarded. For each series of tests a similar number of controls was employed.

Results. Of the 63 mice used as controls, complete remission of the swollen joints occurred in only one case. There was usually an increase in the number of joints involved for a week following the inoculation of the organisms, after which the number remained constant.

When 10,000 units of penicillin G were injected intraperitoneally 3 times a day for 4 days, 12 of the 32 mice (37.5%) made apparent gross recovery. However, 69% of another group of mice which received a smaller dose of penicillin G 3 times a day for 6 days were cured (Table I).

Streptomycin produced even more striking results. There was an 85% recovery in 7 mice when they were given 5,000 units intraperitoneally 3 times a day for 3 days. Of the 17 mice which received 2,500 units 3 times a day for 3 days, 13 lost all signs of previous swelling of their limbs (76%). Because of the unavailability of streptomycin at the time this study was initiated, more test animals could not be employed.

The 2 gold compounds which were studied at various dose levels did not have any effect on the joint involvement in the mice.

All the other substances used were added in various amounts to ground dog chow (Purina) which comprised the diet of the mice. In a group of mice which had been fed 1% sulfathiazole for 38 days none of the animals showed recovery. The level of sulfathiazole used was that suggested by Barlow and Hamburger.[7]

Another series of mice received 0.5% p-aminosalicylic acid in their diet but since so many of the mice died the supplementation was discontinued after 9 days. Again there was no improvement in the mice. After a few days on a diet containing 0.5% promizole in ground dog food, the tails and feet of the mice in Group 9 (Table I) turned blue. The diet was discontinued for a week and then resumed. Not only did the same results recur, but there was such a high percentage of fatality that the mice were taken off the diet. Bambas also observed that both promizole and p-aminosalicylic acid possessed high toxicities for mice.[8] No curative effect was noted when promin in a concentration of 0.5% was included in the diet. This substance was well tolerated by the mice. No improvement was observed when either 0.5% p-aminobenzoic acid or 0.2% methylene blue was added to the diet. The dosage used for these latter agents

[7] Barlow, O. W., and Hamburger, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 317.

[8] Bambas, L. L., personal communication.

was that suggested by McLimans and Grant.[9]

Discussion. Heilman[10] and Brown and Nunemaker[1] have reported the ineffectiveness of sulfonamides in the prevention of fatal infections when *Streptobacillus moniliformis* was inoculated in mice. In the treatment of arthritis in mice produced by a pleuropneumonia-like microorganism, Findlay *et al.*[11] and Sabin and Warren[12] found sulfonamides, as well as arsenicals and salicylates, to be of no value.

Heilman[10] and Brown and Nunemaker[1,13] reported that a single dose of myochrysine would protect mice from infection and death after inoculation with *Streptobacillus moniliformis*. These investigators, however, injected the myochrysine immediately after the microorganisms were inoculated. Brown and Nunemaker found that if they allowed a chronic arthritis to develop in mice, daily subcutaneous injections of myochrysine for a 2-week period had no effect. The present data are in agreement with this last set of results. On the other hand, Findlay[11] and Sabin and Warren[12] showed certain gold compounds to possess prophylactic and curative value in arthritis in mice caused by pleuropneumonia-like organisms. It should be noted, however, that the arthritis produced by these organisms was a non-suppurative type while in the arthritis that develops in mice following the injection of *Streptobacillus moniliformis*, suppuration is a constant finding.

Brown and Nunemaker[1] reported recovery with myochrysine in 2 cases of rat bite fever in human subjects where there was involvement of the joints and *Streptobacillus moniliformis* was isolated. They stated that although improvement following the use of gold therapy was quite definite, it might have occurred spontaneously. Heilman and Herrell[14] reported 100% protection in mice when penicillin was administered subcutaneously beginning 4½ hours after inoculation of their strain of *Streptobacillus moniliformis*. The infected mice continued to receive penicillin at a level of 1,000 units per day for 7 days. But if penicillin was given for only 5 days, 50% of the mice developed swollen joints. In the present study, although treatment was not begun until 3 days after the first appearance of the arthritis, 60% of the mice recovered if 1,000 units were given 3 times a day for 6 days. Thirty-seven per cent were cured if 10,000 units were administered 3 times a day for 4 days.

The use of penicillin in the treatment of infections due to *Streptobacillus moniliformis* has brought about dramatic recoveries in as short a period as 24 hours, with complete disappearance of the arthritis.[15–17] Altmeier *et al.*,[15] Wheeler,[16] and Weber and Favour[17] reported cases where patients with arthritis due to *Streptobacillus moniliformis* infections promptly recovered with daily intramuscular injections of penicillin.

The most effective therapeutic agent in the treatment of the strain of *Streptobacillus moniliformis* used in the present study was streptomycin. Sprecher and Copeland[18] used this drug in one case where there was joint involvement and in which *Streptobacillus moniliformis* was isolated from the blood. The patient had been treated unsuccessfully with penicillin for 6 days before streptomycin was administered. The third dose of the latter antibiotic brought permanent relief. The authors explained the inefficacy of penicillin in this case by stating that the treatment of the infection caused by *Streptobacillus moniliformis* might depend upon the susceptibility or resistance of the strain involved.

Five other agents were treated in the present study in regard to their effect on joint involve-

[9] McLimans and Grant, *Science*, 1947, **105**, 181.

[10] Heilman, F. R., *Science*, 1940, **9**, 366.

[11] Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., *J. Exp. Path.*, 1940, **21**, 13.

[12] Sabin, A. B., and Warren, J., *J. Bact.*, 1940, **40**, 823.

[13] Brown, T. M., and Nunemaker, J. C., *J. Clin. Invest.*, 1940, **19**, 768.

[14] Heilman, F. R., and Herrell, W. R., *Proc. Staff Meet. Mayo Clinic*, 1944, **19**, 259.

[15] Altmeier, W. A., Snyder, H., and Howe, G., *J. A. M. A.*, 1943, **127**, 270.

[16] Wheeler, W. B., *Am. J. Dis. Child.*, 1945, **69**, 215.

[17] Weber, R. A., and Favour, C. B., *Bull. Johns Hopkins Hosp.*, 1945, **77**, 132.

[18] Sprecher, M. H., and Copeland, J. R., *J. A. M. A.*, 1947, **134**, 1014.

ment produced in mice. These substances were investigated because it has been reported that they possess therapeutic activity against some types of microorganisms. The sulfones, promin, and promizole have been reported to be active against some of the acid-fast bacilli.[19-21] P-aminosalicylic acid has also been used to treat experimental tuberculosis.[22] P-aminobenzoic acid has been shown by Snyder *et al.*[23] to be effective in protecting mice infected with a type of epidemic typhus. Methylene blue was shown to be of value in increasing the survival time of mice infected with the etiological agent of Tsutsugamushi disease.[24] None of the above-mentioned agents cured any of the mice of the joint involvement produced by infection with *Streptobacillus moniliformis*.

Summary. A study of the chemotherapy of the arthritis produced in mice by inoculation with a virulent strain of *Streptobacillus moniliformis* was made. Three days after the animals developed joint involvement, therapy was initiated. Under the conditions studied streptomycin was the most effective agent in curing the joint involvement. Penicillin was also effective but to a lesser degree. No cures were obtained with promin, promizole, sulfathiazole, p-aminobenzoic acid, p-aminosalicylic acid, methylene blue, and two gold compounds (Myochrysine and Solgonal-B).

The authors wish to thank Dr. Carl E. Duffy for his advice and assistance during the course of this study.

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[20] Feldman, W. H., Hinshaw, H. C., and Mann, F. C., *Am. Rev. Tuberc.*, 1944, **50**, 418.

[21] Foget, G. H., Pogge, R. C., and Johansen, F. A., *U. S. Public Health Rep.*, 1946, **61**, 957.

[22] Lehman, J., *Lancet*, 1946, **250**, 15.

[23] Snyder, J. C., Maier, J., and Anderson, C. R., Report to Division of Medical Sciences, National Research Council, December 26, 1942.

[24] McLimans, W. F., and Grant, C. W., *Science*, 1947, **105**, 181.

16470 P

Influence of Diet, Sex, and Testosterone Propionate on the Toxicity of Monocrotaline in Rats.*

OSCAR D. RATNOFF AND GEORGE S. MIRICK.

From the Department of Medicine, Johns Hopkins University School of Medicine, Baltimore.

Robertson[1] described the production of hepatic damage in horses fed *senecio*, an observation confirmed in other species.[2-4] Adams and Rogers[5] demonstrated that monocrotaline, an alkaloid derived from *crotalaria*, contained the same active retronecine alkanolamine present in *senecio* alkaloids. Harris, Anderson and Chen[6] observed that mice injected intravenously with monocrotaline died with pulmonary edema, ascites, hydrothorax, and thymic necrosis. There was central hepatic necrosis, hemorrhage and sinusoidal congestion, with little leukocytic reaction. Repeated administration of sublethal doses produced unimpressive hepatic lesions. In

* This work was supported by a grant from the U. S. Navy Department Office of Naval Research.

[1] Robertson, *Cape Agricultural J.*, May, 1906, quoted by 2.

[2] Theiler, A., U. of S. Africa, Department of Agriculture 5th and 6th Reports of the Director of Veterinary Research, April, 1918, p. 7.

[3] Wilmot, F. C., and Robertson, G. W., *Lancet*, 1920, **2**, 848.

[4] Rosenfeld, I., and Beath, O. A., *Am. J. Clin. Path.*, 1945, **15**, 407.

[5] Adams, R., and Rogers, E. F., *J. Am. Chem. Soc.*, 1939, **61**, 2815.

[6] Harris, P. N., Anderson, R. C., and Chen, K. K., *J. Pharmacol. and Exp. Therap.*, 1942, **75**, 78.