16631

Experimental Streptomycin Therapy in Mice.*†

ERNEST JAWETZ.

From the Division of Bacteriology, University of California Medical School, San Francisco.

In contrast to the sulfonamides, penicillin and streptomycin do not require constant significant blood levels for satisfactory therapeutic results. Experiments in streptococcal infections of mice[1] have clearly indicated that the effect of crude penicillin on the bacterial population outlasts measurable blood levels by many hours. Animals were cured of otherwise fatal infections with single large doses or by spacing injections of penicillin 8-24 hours apart.[2,3,4] Clinical experience in pneumococcal[5,6] and streptococcal[7] disease has also demonstrated the effectiveness of dosage regimens by which aqueous penicillin was injected every 8-12 hours. Experiments with streptomycin have given comparable results.[8]

A possible objection to some of the experimental work presented in this field and its translation into clinical terms is the nature of the laboratory model often used for evaluation of chemotherapeutic effectiveness. Animals are given overwhelming infections, usually by "unnatural" routes, and then treated before there is clinical evidence of the disease. The results thus present the effects of chemoprophylaxis rather than chemotherapy. In the course of studies on a pneumotropic pasteurella of mice[9] observations were made which are of some interest in regard to this question.

Methods. The pneumotropic pasteurella used in this work has been described elsewhere.[9] The organism kills mice only if inoculated intracerebrally (LD_{50}: *ca.* 10^4 bacteria) or intranasally (LD_{50}: *ca.* 5×10^5 bacteria). After intracerebral inoculation neurological signs appear within 12-18 hours and all deaths occur in 36-72 hours with a brain abscess. When infected by the respiratory route the mice show cyanosis and dyspnea, with pulmonary consolidation in 18-24 hours and 70-95% die in 2-6 days. The pasteurella is sensitive to streptomycin. It is regularly completely inhibited by 2-4 γ of streptomycin per milliliter of medium.

The standard inoculum in these experiments consisted of 10^6 bacteria in 0.03 ml of dilutions of a 8-12-hour broth culture, injected intracerebrally or intranasally under light ether anesthesia into 21-day-old (10-14 g) white mice of the stock of the National Institute of Health. The animals were observed for 10 days and deaths recorded. At the end of that period the survivors were autopsied and lung lesions were noted. Among the intracerebrally infected animals there were no untreated survivors, and the treated survivors showed no lesions whatever. The results of these treatment experiments are recorded as the ratio of deaths to the total number of animals in the group. Among intranasally infected animals, however, not all untreated mice died and some of the treated survivors showed extensive lung lesions. In these groups the results are therefore expressed as ratio of "infected" animals to the total number in the group. "Infected"

* The work described here was performed at the Division of Infectious Diseases, National Institute of Health, Bethesda, Md.

† The author wishes to thank Doctors M. Finland and E. K. Marshall, who read the manuscripts and made valuable suggestions.

1 Jawetz, E., *Arch. Int. Med.*, 1946, **77**, 1.

2 Zubrod, C. G., *Bull. Johns Hopkins Hosp.*, 1947, **81**, 400.

3 Eagle, H., *Am. Int. Med.*, 1948, **28**, 260.

4 White, H. J., Baker, M. J., and Jackson, E. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 199.

5 Tillet, W. S., Cambier, M. J., and McCormack, J. E., *Bull. N. Y. Acad. Med.*, 1944, **20**, 142.

6 Marshall, E. K., and Zubrod, C. G., Personal communication.

7 Jawetz, E., *Arch. Int. Med.*, 1948, in press.

8 Zubrod, C. G., *Bull. Johns Hopkins Hosp.*, 1948, **82**, 357.

9 Jawetz, E., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 46.

TABLE I.
Streptomycin Treatment of Intracerebral Pasteurella Infection.
Mice infected with 100 LD_{50} intracerebrally given 3 subcutaneous injections of 0.1 g/kg streptomycin each, at various intervals.

Treatment time, hr after infection	Deaths/total	% survivors (cured)
2, 6, 24	2/12	83.3
2, 24, 48	1/12	91.6
6, 10, 24	6/12	50.0
6, 24, 48	8/12	33.3
10, 24, 48	12/12	0.0
Controls (untreated)	24/24	0.0

refers to both the dead animals and those which survived but had gross lesions in the lungs.

The streptomycin (a solution with an assay value of 200 mg streptomycin base/ml) was kindly supplied by Dr. H. Welch, Food and Drug Administration, Washington, D.C. Solutions of the required strength were prepared in physiological saline and were injected subcutaneously in amounts of 0.2 ml per dose as indicated in the various dosage schedules.

Results. Table I summarizes selected results of preliminary trials in the treatment of intracerebral infections by subcutaneously administered streptomycin. With fixed individual and total dose the outstanding factor which determined success of therapy was the interval between the injection of bacteria and the first administration of streptomycin. It was immaterial whether the second dose was given 4 or 22 hours after the first. If the total dose exceeded 0.3 g/kg and the first of 3 injections was given not later than 4 hours after intracerebral infection at least 75% of the animals could be cured. For therapy to be successful it had to be started long before neurological symptoms appeared.

When identical treatment schedules were applied to groups of mice infected intracerebrally or intranasally, the milder respiratory infection responded much more readily. With the first dose of antibiotic administered to all mice 4 hours after infection, there was little difference between the results of various dosage schedules (Table II). The ED_{50} (dose resulting in 50% cure)[10] for cure of infection of the central nervous system ranged between 71 and 108 mg/kg with the various regimens, and it was approximately half of those values for the pulmonary disease. These results considered by themselves might indicate similar response of the "artificial" (intracerebral) and "natural" (respiratory) infection to streptomycin treatment. It must be considered, however, that treatment 4 hours after infection is relatively early for the latter and late for the former.

Good evidence, relating the time elapsed before treatment was started to the therapeutic results, was obtained in the comparative tests illustrated in Table III. If treatment was started 4 hours after infection, when neither symptoms nor lesions were present, a number of schedules of therapy were satisfactory. There was no marked difference between total doses of 0.05 g/kg and 0.3 g/kg whether administered in a single dose or in three injections 2 or 24 hours apart. If, however, 18 hours were permitted to elapse between infection and first treatment, statistically significant differences appeared between the results of various low-dose schedules. With a total of 0.1 g/kg, the single dose was quite unsatisfactory, whereas with 3 doses administered at 2-hour intervals, only one-fourth of the animals showed lesions. Three injections 24 hours apart gave intermediate results. These differences between various dosage schedules were not apparent at higher total dose levels (Table III). In order to obtain satisfactory response with long-interval regimens, the dosage had to be larger than with schedules employing injections every 2-4 hours.

Comment. Experimental work in chemotherapy is often based on overwhelming types of infection. Small numbers of highly virulent organisms are introduced into animals in such a manner as to permit rapid multiplication and to produce prompt death. Thus the animals represent little more than living test-tubes to which chemotherapeutic agents are added early in the infection, prior to the appearance of symptoms and lesions. Under

[10] Quan, S. F., Foster, L. E., Larson, A., and Meyer, K. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **66**, 528.

TABLE II.
Comparison of Treatment Results with Various Dosage Schedules in Intranasal and Intracerebral Pasteurella Infection.

Total dose streptomycin g/kg	Intranasal infection: Treatment Schedules. Single inj.	3 inj. q 2 h	3 inj. q 8 h	3 inj. q 24 h	Intracerebral infection: Single inj.	3 inj. q 2 h	3 inj. q 8 h	3 inj. q 24 h
.05	9/20*	5/20	4/19	5/20	16/20†	14/20	17/20	15/20
.20	4/30	2/30	3/29	4/30	8/20	5/20	2/20	10/20
.30	4/30	1/28	3/30	3/30	7/20	1/20	1/19	6/20
.40	—	2/20	1/18	1/20	—	0/19	0/20	1/20
0	46/50	—	—	—	60/60	—	—	—

All mice infected with 10^6 bacteria. First dose of antibiotic given 4 hr after infection.
* Infected/Total.
† Deaths/Total.

TABLE III.
Influence on Therapeutic Results of the Time Elapsed Before Treatment Was Started. Total Dose of Streptomycin.*

Treatment started	Dosage schedule	0.1 g/kg Inf./Tot.	0.1 g/kg % lesions	0.3 g/kg Inf./Tot.	0.3 g/kg % lesions	ED_{50} g/kg	S.E. of ED_{50} g/kg
4 hr after infection	Single dose	13/40	32.4	4/10	10.0	.081	± .013
	3 × q2h	8/39	20.5	1/40	2.5	.052	± .009
	3 × q24h	7/40	17.2	3/38	7.9	.063	± .011
18 hr after infection	Single dose	35/40	87.5	12/40	30.0	.219	± .028
	3 × q2h	11/40	27.5	8/40	20.0	.048	± .020
	3 × q24h	19/39	48.6	9/40	22.5	.106	± .014
Controls	0	49/52	94.2	—	—	—	—

All mice infected with 10^6 pasteurella.
ED_{50}: Dose resulting in 50% freedom from lesions. S.E. of ED_{50}: Standard error.[10]
* Only 2 doses are given here. Additional ones were used to calculate ED_{50} and its standard error.

such conditions the natural defense mechanisms of the host do not commonly come into play and the animals die from intoxication or organ necrosis, often without the development of gradually progressive lesions.

In a few human infections, like pneumonic plague, the pathogenesis parallels that seen in experimental animals,[11] and experimental chemotherapeutic results may be directly applicable to patients. In the majority of human infections, however, the disease is much more complex than that of the "test-tube" experiment in animals. Host reactions influence the development of tissue lesions which may have a spontaneous tendency toward healing, and antibiotics probably support mechanism of defense rather than replace them. In human disease chemotherapy is seldom started prior to the development of symptoms or lesions. The extent to which results of "test-tube" experiments in animals mirror events in patients is debatable.

The observations reported in this paper indicate that route of inoculation, nature of disease, and the time treatment is started, can influence considerably the relative merits of various dosage schedules employed in chemotherapy.

Intracerebral pasteurella infection can be cured by streptomycin, subcutaneously administered to mice. This in itself is a remarkable finding. The data cannot be used to support the opinion[12] that some types of human central nervous system infections like-

[11] Litchfield, J. T., and Fertig, J. W., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 276.

[12] Hoyne, A. L., and Brown, R. H., *J.A.M.A.*, 1948, **136**, 597.

wise are cured by intramuscular streptomycin alone. The intracerebral pasteurella infection of mice is strictly of the "test-tube" type and treatment is successful only if started prior to the appearance of symptoms.

Mice infected with the same pasteurella by the "natural" respiratory route suffer from a slower, progressive disease and die usually from anoxia due to widespread pneumonic lesions. Streptomycin is effective when started relatively late in that disease process, but even high doses may cure only a certain proportion of the animals. Such late treatment emphasized differences between dosage schedules, not apparent under less critical conditions. The use of similar "natural" types of infection in chemotherapeutic testing is not likely to yield as clear-cut pharmacological results as the "test-tube" types but it might contribute information on the dynamics of antibiotic action important in human disease.

Summary. 1. The therapeutic effect of streptomycin was evaluated in mice infected with a pneumotropic pasteurella either by the respiratory or by the intracerebral route.

2. When treatment was started within 4 hours after infection intracerebrally mice could be saved by streptomycin injected subcutaneously. With a total dose of 0.3 g/kg streptomycin the main factor determining success of therapy was the time interval between infection and first administration of antibiotic.

3. With comparable dosage regimens larger amounts of streptomycin were necessary to cure the intracerebral than the respiratory infection.

4. When treatment of the pulmonary infection was delayed for 18 hours, until symptoms and lesions were present, marked differences appeared in the effectiveness of dosage schedules similar in their effects when treatment was started soon after infection.

5. The possible usefulness of "natural" type infections in chemotherapeutic experiments is discussed and compared to the "test-tube" type of experimental disease.

16632

Failure of an Extract of Pregnant Mares' Urine to Influence Gastric Secretion in Man.

JOSEPH B. KIRSNER, ERWIN LEVIN, AND WALTER LINCOLN PALMER.

From the Frank Billings Medical Clinic, Department of Medicine, University of Chicago.

The effect of extracts of urine upon gastric secretion and upon the course of experimentally induced ulcer has been studied by many investigators. Sandweiss, Saltzstein, and Farbman[1,2] noted delay or prevention of ulcers in the dog following the administration of extracts prepared from human pregnancy urine and the urine from normal women. Various observers[3-9] have reported the presence in human and canine urine of a substance inhibiting gastric motility and the secretion stimulated by histamine and by a meal. Fur-

1 Sandweiss, D. J., Saltzstein, H. C., and Farbman, A., *Am. J. Dig. Dis.*, 1938, **5**, 24.

2 Sandweiss, D. J., Saltzstein, H. C., and Farbman, A., *Am. J. Dig. Dis.*, 1939, **6**, 16.

3 Friedman, M. H. F., Recknagel, R. O., Sandweiss, D. J., and Patterson, T. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 509.

4 Necheles, H., Hanke, M. E., and Fantl, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 618.

5 Gray, J. S., Wieczorowski, E., and Ivy, A. C., *Science*, 1939, **89**, 489.

6 Culmer, C. U., Atkinson, A. J., and Ivy, A. C., *Endocrinology*, 1939, **24**, 631.

7 Gray, J. S., Wieczorowski, E., Culmer, C. U., and Adkinson, J. L., *Am. J. Physiol.*, 1940, **129**, 589.

8 Culmer, C. U., Gray, J. S., Adkinson, J. L., and Ivy, A. C., *Science*, 1940, **91**, 147.

9 Friedman, M. H. F., and Sandweiss, D. J., *Am. J. Dig. Dis.*, 1941, **8**, 366.