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TREATMENT OF BUBONIC PLAGUE WITH SULFONAMIDES AND ANTIBIOTICS

SIR SAHIB SINGH SOKHEY, M.A., M.D., F.N.I.
Formerly Director, Haffkine Institute, Bombay, India

P. M. WAGLE, M.D., D.P.H.
Director, Haffkine Institute, Bombay, India

M. K. HABBU, B.Sc.
Assistant Bacteriologist, Department of Vaccines, Haffkine Institute, Bombay, India

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SYNOPSIS

To assess the curative value of different drugs in bubonic plague infection, white mice were infected in the laboratory with living *Pasteurella pestis*, and the treatment with the drug to be tested was begun either 48 or 72 hours after infection, it taking 48-72 hours for the development in mice of septicaemia—the decisive factor in plague infection. Sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfadiazine, antiplague serum, penicillin, streptomycin, aureomycin, chloramphenicol, and oxytetracycline were tested. Sulfapyridine and penicillin gave no protection, but the remainder had a curative effect in 50% or more of the animals. The antibiotics, in particular, with the exception of penicillin, protected 90%-100%.

Trials were then carried out on humans under controlled conditions in the field—antiplague serum, sulfapyridine, sulfathiazole, sulfamerazine, sulfadiazine, and streptomycin being tested. While there were no statistically significant differences between the results obtained with the various drugs, the clinical effect of streptomycin was striking. In all cases treated with this substance the temperature reverted to normal earlier than with sulfadiazine or sulfamerazine, and in septicaemic cases it became normal in 50 hours, on the average, against 85 hours and 89 hours for sulfadiazine and sulfamerazine respectively.

Laboratory Trials

Assessment in the laboratory of the curative value of any drug in plague infection is a comparatively easy matter. Plague is essentially a disease of rodents and only incidentally spreads to human beings. We have, in the white mouse, a very susceptible animal in which the disease can be

induced, producing an infection which is in every way similar to human infection. For this reason the value of the drug can be assessed by standardized methods in the laboratory, and experience has shown that the laboratory results closely correspond to the results of the field trials in human beings.

Until 1931 the only remedies which were tried in the treatment of plague were iodine, mercurochrome, and some others, which produced little or no effect. In 1931, Naidu [1] prepared, in buffaloes, his antiplague serum, which, when tried in bubonic cases, gave encouraging results. Later, Sokhey [3, 4] produced a potent antiplague serum by the hyperimmunization of horses.

Though a new era in chemotherapy was started in 1935 by the announcement of sensational results by Domagk, it did not find its application in the treatment of plague until 1939, when Schütze [2] showed that sulfapyridine was effective in the treatment of experimental plague infection in rats.

Sokhey & Dikshit,[5] in 1940, developed a laboratory method by which the chemotherapeutic effect of various drugs in experimental plague infection in mice could be accurately assessed. The method in brief is as follows.

The Haffkine Institute inbred white mouse was used as the experimental animal. The animals used were 8-9 weeks old and weighed about 25-30 g. This strain is uniformly and constantly susceptible to plague, and 6-12 organisms of a fully virulent strain of *Pasteurella pestis* injected subcutaneously invariably produce 100% mortality. As an infective dose in these tests 75-150 organisms per animal were given.

The drug under test was made into an emulsion in 10% gum-acacia solution so that 0.5 ml of the emulsion contained the selected dose of the drug, and was introduced into the stomach by means of a pipette.

In plague infection, the most important factor which decides the issue is the development of septicaemia. In mice, under the conditions obtaining in these experiments, septicaemia develops 48-72 hours after the infection has been induced. In experimental animals, therefore, treatment was started in one batch 48 hours after inducing the infection and in another batch 72 hours after inducing the infection. Antiplague serum, penicillin, and streptomycin were given subcutaneously, the selected doses being contained in 0.2 ml of the solution. The results obtained are shown in table I.[5, 9]

Although the results are for the administration of the selected sulfonamides in a dose of 20 mg twice a day for 10 days in the case of sulfapyridine and sulfathiazole and 5 mg four times a day for 5 days in the case of sulfadiazine, sulfamerazine, and sulfamethazine, higher and lower doses were tried for varying periods. Only those schedules giving the optimum effect of the drug are given in table I.

These results clearly show that sulfapyridine and penicillin gave no protection, whereas antiplague serum, sulfathiazole, sulfamerazine, sulfa-

TABLE I. SCHEDULES OF TREATMENT OF PLAGUE-INFECTED MICE WITH VARIOUS DRUGS

Drug	Infective dose of *Pasteurella pestis* (number of organisms)	Treated infected mice		Untreated infected mice (control)		
		number used	number dead	number used	number dead	average survival time (days)
Time of treatment: 48 hours after infection Dosage: 20 mg twice a day for 10 days						
Sulfathiazole	80	10	1	10	10	5.7
Sulfapyridine	132	10	10	10	10	5.6
Time of treatment: 72 hours after infection Dosage: 20 mg twice a day for 10 days						
Sulfathiazole	146	10	3	10	10	5.7
Sulfapyridine	132	10	10	10	10	4.9
Time of treatment: 48 hours after infection Dosage: 5 mg four times a day for 5 days						
Sulfadiazine	144	10	5	12	12	5.7
Sulfamerazine	144	10	3	12	12	5.7
Sulfamethazine	144	10	3	12	12	5.7
Time of treatment: 72 hours after infection Dosage: 5 mg four times a day for 5 days						
Sulfadiazine	144	10	6	12	12	5.7
Sulfamerazine	144	10	3	12	12	5.7
Sulfamethazine	144	10	3	12	12	5.7
Time of treatment: 72 hours after infection Dosage: 0.1 ml daily for 4 days						
Antiplague serum *	108	10	1	10	10	5.7
Time of treatment: 48 hours after infection Dosage: 1,000 units four times a day for 7 days						
Penicillin **	142	10	10	10	10	5.7
Time of treatment: 48 hours after infection Dosage: 0.8 mg four times a day for 2 days and then 0.2 mg four times a day for 5 days						
Streptomycin **	142	10	0	10	10	5.5
Time of treatment: 72 hours after infection Dosage: 0.8 mg four times a day for 2 days and then 0.2 mg four times a day for 5 days						
Streptomycin **	134	10	1	10	10	6.0

*** The first dose of antiplague serum was given intravenously and the subsequent doses subcutaneously.**

**** Penicillin and streptomycin were given subcutaneously.**

methazine, and streptomycin showed remarkable curative action. However, streptomycin seems to be by far the best drug in the treatment of experi-

mental infection, since 90%-100% of the animals can be saved even when the drug is administered after septicaemia has developed, as against 50%-70% with the sulfonamides.

During the years 1949-50, three more antibiotics which were effective against Gram-negative organisms, and were therefore expected to possess antibiotic effects against plague organisms, were tested for their curative value in experimental plague infection in mice. The results of these tests are shown in table II.[6, 12]

TABLE II. SCHEDULES OF TREATMENT OF PLAGUE-INFECTED MICE WITH AUREOMYCIN, CHLORAMPHENICOL, AND OXYTETRACYCLINE

Drug *	Dose (mg)	Infective dose of *Pasteurella pestis* (number of organisms)	Treated infected mice		Untreated infected mice (control)	
			number used	number dead	number used	number dead
Aureomycin	1.0	98	10	1	10	10
Chloramphenicol	8.0	150	10	1	10	10
Oxytetracycline	8.0	174	10	1	12	12

*** All drugs were given by mouth every 3 hours during the first 12 hours and every 6 hours thereafter for a total period of 10 days.**

The total dose of aureomycin was 42 mg, while in the case of chloramphenicol and oxytetracycline it was 336 mg. If the antibiotics were not given either in sufficient quantity or for a sufficient number of days, infection reappeared within a few days after an apparent cure.

Field Trials

The results obtained with the sulfa drugs and streptomycin in experimental plague infection greatly encouraged us to try out these drugs in actual human cases whenever opportunities were available, and special units were sent under Dr. Wagle to try them under controlled conditions.[7-11, 13] The following were the methods employed.

Antiplague serum. Forty millilitres of serum were given on the day of admission, 20 ml intravenously immediately on admission, and 20 ml subcutaneously 6 hours later. The same quantity of serum was given the next day in a like manner. Subsequent dosage and duration of treatment depended upon the severity of the case. Mild cases did not require any serum after the first two days, but for severe cases it was usually administered for five days.

Sulfapyridine and sulfathiazole. 10-14 g were given on the first day, and 6 g on subsequent days for a maximum period of 10 days, the duration

depending on the severity of the case. The drug was stopped earlier if the temperature became normal or the patient showed distinct improvement in general condition. To comatose patients or when the drug produced persistent vomiting, the drug was given intramuscularly or intravenously.

Iodine. The controls received the usual hospital treatment, i.e., iodine solution intravenously.

The following results were obtained : *

Drug	*Number of cases*	*Number of deaths*	*Case mortality (%)*
Antiplague serum	157	37	23.5
Sulfapyridine (M & B 693)	122	33	27.0
Sulfathiazole	274	59	21.5
Iodine solution (controls)	149	80	53.6

It has already been noted that the most important factor which decides the issue in human plague is the development of septicaemia. If the lymph glands prevent the spread of infection to the blood stream and the infection remain localized, spontaneous recovery almost invariably results. On the other hand, if the organisms pass the lymph glands and septicaemia results, death invariably follows unless an effective agent is administered to control the infection before the latter has caused widespread destruction of the tissues. Thus a better picture of the curative value of the drug will be obtained from the cases where the drug administration was started after the septicaemia had set in. For this purpose, these results have been tabulated separately as follows :

Drug	*Number of cases*	*Number of deaths*	*Case mortality (%)*
Antiplague serum	71	36	50.7
Sulfapyridine (M & B 693)	62	31	50.0
Sulfathiazole	119	50	42.0
Iodine solution (controls)	75	68	90.7

During the years 1942-8, the newer sulfonamides and streptomycin were tested for their curative value in field trials carried out at Latur, Poona, Manchar, and Calcutta. In these cases the dosage was slightly altered to give more adequate concentration of the drug with serum. The following dosage was used :

Sulfadiazine. An initial dose of 4 g was followed by 2 g four hours later. Thereafter, 1 g was given every 4 hours until the patient's temperature remained normal for 2 days. This dosage maintained a concentration of between 10 mg and 20 mg per 100 ml of blood.

Sulfamerazine. An initial dose of 4 g was followed by 2 g four hours later. Then 1 g was given every 8 hours until the temperature remained

* The figures given in all the following tabulations are pooled figures obtained in the different field trials carried out between 1941 and 1948.

normal for 2 days. This dosage maintained a concentration of between 10 mg and 20 mg per 100 ml of blood.

Streptomycin. In relatively mild cases, an initial dose of 2/3 g was followed by 1/3 g every 4 hours until the temperature remained normal for 24 hours. In severe cases, 2/3 g of the drug was given every 4 hours until the temperature remained normal for 2-3 days.

The following results were obtained :

Drug	*Number of cases*	*Number of deaths*	*Case mortality (%)*
Streptomycin	148	6	4.2
Sulfadiazine	180	16	8.9
Sulfamerazine	113	9	8.0

Tabulating these figures separately for the cases with septicaemia at the time of treatment, the following data are obtained :

Drug	*Number of cases*	*Number of deaths*	*Case mortality (%)*
Streptomycin	37	4	10.8
Sulfadiazine	62	13	21.0
Sulfamerazine	22	7	31.8

The results obtained with different treatments may not show statistically significant differences, but the effect of streptomycin was striking. In both non-septicaemic and septicaemic cases, the temperature in cases treated with streptomycin became normal earlier than in cases treated with sulfadiazine or sulfamerazine. In septicaemic cases treated with streptomycin, the temperature became normal in 50 hours on an average, while it took 85 hours with sulfadiazine and 89 hours with sulfamerazine. It is proposed to try out the newer antibiotics—aureomycin, chloramphenicol, and oxytetracycline—whenever suitable opportunities arise.

RÉSUMÉ

L'infection pesteuse de la souris blanche est, sous maints rapports, semblable à celle de l'homme. Aussi est-ce sur cet animal que peuvent être mises à l'épreuve les substances proposées pour le traitement de la peste humaine. Les résultats des essais thérapeutiques de masse ont prouvé le bien-fondé de cette généralisation.

Jusqu'en 1931, on employait comme remèdes contre la peste l'iode, le mercurochrome et quelques autres substances, quasi inefficaces. A cette date, Naidu prépara sur le buffle du sérum antipesteux qui donna des résultats encourageants dans le traitement de la forme bubonique. Sokhey mit au point, par la suite, un sérum très actif produit par hyperimmunisation du cheval. En 1940, Sokhey et Dikshit élaborèrent une méthode d'essai de substances antipesteuses sur la souris blanche (souche du Haffkine Institute) : 75-150 bacilles pesteux sont injectés à chaque souris par voie sous-cutanée ; la septicémie se déclare 48-72 heures plus tard. Les médicaments — sulfamides, antibiotiques et sérum —, émulsionnés dans une solution de gomme acacia, sont administrés 48-72 heures après

l'inoculation bacillaire, le sérum antipesteux, la pénicilline et la streptomycine par voie sous-cutanée, les autres par voie buccale.

Les résultats ont montré clairement que la sulfapyridine et la pénicilline n'assurent aucune protection, tandis que le sérum antipesteux, le sulfathiazol, la sulfaméthazine et la streptomycine ont des propriétés thérapeutiques remarquables. La streptomycine paraît être le médicament de beaucoup le plus efficace dans le traitement de l'infection expérimentale : 90-100 % des animaux peuvent être sauvés, même si le médicament a été administré alors que la septicémie était déjà déclarée (ce chiffre est de 50-70 % avec les sulfamides).

Le succès des expériences sur la souris a engagé les auteurs à procéder à des traitements sur l'homme au moyen des mêmes substances — appliquées selon une posologie décrite en détail dans l'article. Les taux de létalité observés chez les groupes de sujets traités par les divers médicaments n'ont pas présenté entre eux de différences statistiquement significatives ; cependant, l'effet de la streptomycine a été particulièrement frappant : dans les cas septicémiques, comme dans les autres, la température devint normale après 50 heures (85 heures avec les sulfamides).

D'autres antibiotiques seront essayés sitôt que les circonstances le permettront.

REFERENCES

1. Naidu, B. P. B. & Mackie, F. P. (1931) *Lancet*, **2**, 893
2. Schütze, H. (1939) *Lancet*, **1**, 266
3. Sokhey, S. S. (1936) In : *Report of the Haffkine Institute for the years 1932-35*, Bombay, p. 73
4. Sokhey, S. S. (1937) *Anti-plague serum.* In : *Report of the Haffkine Institute for the year 1936*, Bombay, p. 39
5. Sokhey, S. S. & Dikshit, B. B. (1940) *Lancet*, **1**, 1040
6. Sokhey, S. S. & Habbu, M. K. (1950) *Indian J. med. Res.* **38**, 197
7. Sokhey, S. S. & Wagle, P. M. (1943) In : *Report of the Haffkine Institute for the years 1940-1941*, Bombay, p. 37
8. Sokhey, S. S. & Wagle, P. M. (1945) *Sulphadiazine and sulphathiazole in the treatment of bubonic plague.* In : *Report of the Haffkine Institute for the years 1942 and 1943*, Bombay, p. 36
9. Sokhey, S. S. & Wagle, P. M. (1948) *Sulphonamides and antibiotics in the treatment of plague.* In : *Proceedings of the Fourth International Congresses on Tropical Medicine and Malaria, Washington, D.C.*, vol. 1, p. 276
10. Sokhey, S. S. & Wagle, P. M. (1949) In : *Report of the Haffkine Institute for the years 1947 and 1948*, Bombay, p. 49
11. Wagle, P. M. (1944) *Indian med. Gaz.* **79**, 585
12. Wagle, P. M. & Habbu, M. K. (1952) *Terramycin in the treatment of experimental plague infection.* In : *Report of the Haffkine Institute for the year 1950*, Bombay, p. 40
13. Wagle, P. M., Sokhey, S. S., Dikshit, B. B. & Ganapathi, K. (1941) *Indian med. Gaz.* **76**, 29