

- (4) Stems of *Acorus calamus* (N.O. Araceæ).
- (5) Leaves and stems of *Aristolochia bracteata* (N.O. Aristolochiaceæ).
- (6) Seeds of *Butea frondosa* (N.O. Papilionaceæ).
- (7) Flowers and leaves of *Spilanthes acmella* (N.O. Compositæ).

The first four plants had either no lethal effect or were feeble in action. Durranta is a commonly used hedge-plant and Dr. Manson¹ suggested that the alkaloid in the berries was possibly the toxic factor. We have extracted the alkaloid and found it to be possessing a negligible toxicity.

Aristolochia bracteata and *Butea frondosa* showed a fairly good action. The preliminary trials with alcoholic and water extracts of *Butea frondosa* having proved rather encouraging the petrol extract was tried and was found to be effective in a concentration of 1 per cent. in water.

Spilanthes acmella.—The fresh flowering tops of this plant were extracted with ether. The ether extract was yellowish in colour and produced a highly tingling sensation on the tongue. The extract is insoluble in water but is easily soluble in boiling alcohol, cold benzene or solvent naphtha. The larvicidal property of the extract was studied quantitatively as follows: 100 mgs. of the extract was dissolved in 5 c.c. of alcohol with 100 mgs. of pure castile soap and this solution was poured into one litre of water with shaking. The suspension of the extract thus formed, was stable enough for the work. This suspension was diluted with the required quantities of water to give the different concentrations used in the experiments. Controls, containing equivalent quantities of alcohol and soap in the same concentrations used, were also kept. These controls did not show any effect on the larvæ over 24 hours of observation. These suspensions and controls were kept in beakers and 10 anopheline larvæ were kept in each. All the larvæ used in the experiment were fresh, obtained from the same habitat and were of the third or the fourth stage. The larvæ in suspensions of the material, first showed a stage of irritation with brisk movements, later on lost their activity and were unable to reach the surface. The time was noted when all the larvæ lost their capacity to reach the surface and is indicated in the following table under the heading as "The time required for the loss of capacity to float". The time taken by all the larvæ for complete death is also noted and is given in the table. These experiments were repeated several times with closely similar results. It is evident from these experiments that the extract of *Spilanthes acmella* is lethal to anopheline larvæ even in a dilution of 1 in 100,000.

TABLE I

Concentration	Time required for the loss of capacity to float	Time required for death
1 part in 10,000 of water	5 minutes	40 minutes
1 " " 25,000 "	15 "	120 "
1 " " 50,000 "	25 "	180 "
1 " " 100,000 "	35 "	15 hrs.

Chemical investigation of this plant is in progress and spilanthol² has been isolated and identified in the ether extract. Spilanthol was isolated earlier by Japanese workers from *Spilanthes oleracea* or American para-cress. The ether extract on treatment with 60 per cent. alcohol precipitates a large amount of waxes and sterols which have no action on larvæ and also have no tingling taste. The alcohol-soluble material has a tingling taste and about two-thirds of it is spilanthol, which appears to be the main active constituent.

A patent regarding the use of this extract as larvicide has been applied for.

We take this opportunity to express our heartfelt thanks for the supply of flowers to Prof. L. S. S. Kumar, Economic Botanist to the Government of Bombay, without whose help and co-operation it would have been impossible to carry out this work.

Further work is in progress.

G. S. PENDE,
N. L. PHALNIKAR
B. V. BHIDE.

Maharaja Pratapsinh Chemical Lab.,
Sir Parashurambhau College, Poona,
and

Indian Drugs Res. Assn., Poona,
December 6, 1944.

1. Manson, J. *Malaria Inst.* (Delhi), 1930, 2, No. 1, 85. 2. Gerber, F., *Arch. Pharm.*, 1903, 241, 270. V. Asahina and M. Asano, *J. Pharm. Soc.* (Japan), 1920, 503. —, *Ibid.*, 1922, 85. M. Asano and T. Kanematsu, *Ber.*, 1932, 65 (B), 160.

ACTIVITY OF SULPHANILYL- BENZAMIDE AGAINST TYPE I PNEUMOCOCCAL INFECTION IN MICE (A Preliminary Note)

IN a previous paper (Bose and Ghosh, 1944) it has been noted that after oral administration in mice, sulphanilyl-benzamide gave rise to a fairly high blood concentration with low percentage of conjugation, and maintained a more steadier level than sulphanilamide. Further work on its urinary excretion (Bose and Ghosh, 1944) in human volunteers, has also shown it to be rapidly eliminated through the system. Brownlee and Tonkin (1943) have already observed its bacteriostatic effects *in vitro* against the intestinal pathogens. Considering its high systemic absorption and rapid urinary excretion, it was considered to be of interest to study its effect against certain coccal infections of the body. The present paper deals with the result of treatment by sulphanilyl-benzamide against Type I pneumococcal infection in mice.

EXPERIMENTAL

The technique employed in assessing the therapeutic activity of the drug was essentially the same as described by Bose *et al.* (1941) in a similar paper on sulphamethylthiazole. The drug was fed to mice in a 5 per cent. aqueous solution of pH ca 8.2. The animals used weighed 20 to 22 gms. The activity of the drug was compared simultaneously with that of 2-(p-amino-benzene sulphonamido)-pyridine, which was taken as a standard. Two

Effect of treatment of sulphanilyl-benzamide and sulphapyridine on mice (average weight 20 gms.) infected with pneumococcus Type I

Infecting dose = 0.2 c.c. of 10^{-6} dilution of an 18-hour serum broth culture.
Number of mice in each group = 20.

Days of observation after infection	Group A, control		Group B, Sulphapyridine		Group C, Sulphanilyl benzamide			Group D, Sulphanilyl-benzamide			
	Dead	Survived	Daily dose fed	Dead	Survived	Daily dose fed	Dead	Survived	Daily dose fed	Dead	Survived
1	0	20	20 mg.	0	20	20 mg.	0	20	30 mg.	0	20
2	10	10	20 "	0	20	20 "	0	20	30 "	0	20
3	14	6	20 "	1	19	20 "	6	14	30 "	6	14
4	6	0	20 "	6	14	20 "	18	2	30 "	6	14
5				6	14		19	1		10	10
6				7	13		20	—		16	4
7				9	11					17	3
8				11	9					17	3
Average survival days (max. 8)	1.8		6.0		2.85			4.4			

sets of doses, once daily, were employed in the case of sulphanilyl-benzamide and a single set in case of sulphapyridine. The drug given at each administration and the number of animals used with each dose are stated in their respective protocols.

The organism for producing the infection was a strain of Type I pneumococcus, the virulence of which was maintained by repeated passage in mice. Previous to experiment, the virulence of the strain was so much enhanced that 0.2 c.c. of a 10^{-6} dilution was sufficient to kill the animals in 48 hours. This was taken to be the minimum lethal dose. The dose used for infecting the animals was 0.2 c.c. of 10^{-6} dilution of an 18-hour serum broth culture, which contained about 1,000 M.L.D.s. The animals were injected intraperitoneally with the infecting dose half an hour before commencing the treatment. The observation was continued for a period of eight days, and the animals, dying on a day, were all subjected to post-mortem examinations. Cultures of heart blood in all these animals were positive for pneumococci. The average survival time as given in the table, was calculated by totalling the number of days survived by each mouse and then dividing by the number of animals used in each investigation according to the procedure followed by Whitby (1937).

DISCUSSION AND CONCLUSION

From a statistical analysis of the average survival time and the mortality rates (*vide* Table) it is evident that the lower dosage of sulphanilyl-benzamide does not give any significant protection to mice infected with a highly virulent strain of pneumococcus Type I. But, on the other hand, it is being found that the difference between the survival time of mice treated with the higher dosage (30 mgs. daily) and that of the untreated controls is certainly significant. This is also apparent from the fact

that a certain number of mice were fully protected up to the period of observation with this dose, while there were none with the lower one. Of course, the drug is far behind in activity when compared with sulphapyridine, where a higher rate of average survival time and a larger number of fully protected animals are found. It can, thus, be concluded that contrary to sulphanilamide which has no protective action against Type I pneumococcus, sulphanilyl benzamide possesses a definite anti-pneumococcal activity, though inferior to sulphapyridine.

It remains, however, to be seen whether this widening of activity possessed by this substituted sulphanilamide derivative is in any way directly related to the substitution of So, NH, radicle; or whether the breaking up of the substituted molecule inside the body into p-amino-benzene-sulphonamide would be the cause of such activity.

SUMMARY

1. Sulphanilyl-benzamide, when administered in higher dosage (30 mgs. daily for four days) to mice, infected with pneumococcus Type I, possesses a definite anti-pneumococcal activity as shown by an increase in survival period and a certain percentage of fully protected animals.

2. The activity of the drug, in comparison with sulphapyridine, however, is less.

Bengal Immunity Research Lab.,
Calcutta,
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A. N. BOSE.
J. K. GHOSH.

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