a result of which the incidence of relapse is likely to be increased. Aureomycin is another antibiotic which is reported to have some place in the treatment of enteric fever. It is stated that Chloramphenicol does not appear to be excreted in a biologically active form in the bile (Herrell, 1951). It may be due to this fact that the treatment of typhoid carriers has been uniformly unsuccessful with Chloramphenicol (Douglas, 1950; Ranball & Mose, 1949; Woodward *etal*, 1950). On the other hand Aureomycin is claimed to be peculiarly adapted to the treatment of biliary infection because of the high concentration reached by it in the bile (Strax & Wright, 1949). A synergism may exist between these two antibiotics in the treatment of enteric fever. Further studies will prove or disprove this assumption.

The results obtained in this small series are encouraging and deserve further trial and fuller study.

*Summary.*

A series of 22 cases of enteric fever treated with combined Chloramphenicol and Aureomycin is reported.

Results have been found to be encouraging.

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## TESTING OF INSOLUBLE DRUGS AND OILS FOR THEIR BACTERICIDAL PROPERTIES

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TESTING of the drugs and oils insoluble in water for their bactericidal activities present a problem which has not been satisfactorily solved. Bhatnagar and Fernandez (1950) used 2 per cent gum arabic culture media for formosulphathiazole and similar drugs and Mackenzie *et al.* (1948) used 3 per cent ethyl-alcohol for dissolving the insoluble drugs before adding to their synthetic media. With both the above methods the drug does get uniformly distributed in the media but gets ultimately precipitated if it is highly insoluble. The drugs reported in this paper are mostly insoluble in water beyond a dilution of 1 in 10,000 and are soluble in alcohol in a minimum of 90 per cent alcohol. The solubilities of these drugs beyond 1 in 10,000 were not determined. No work appears to have been published on the testing of oils for their bactericidal properties and a method which has been worked out and found satisfactory is reported in this paper.

*Experimental*

*Testing of insoluble drugs against Myco tuberculosis.*—Fifteen milligrammes of the drug accurately weighed are finely powdered in a sterilized glass pestle and mortar and thoroughly mixed with 15 cc. of Lownenstein Jensen medium added drop by drop initially. Five cubic centimeters of this medium containing 1 in 1,000 of the drug are thoroughly mixed with 15 cc. of the medium. Five cubic centimeters each are transferred to two sterlized test tubes, and another five cubic centimetres left in the mortar and the rest thrown away. In this way dilutions of the drug up to 1 in 64,000 or more are made in Lowenstein medium. Changes in the drugs in the alimentary canal of animals if any were simulated by digesting them consecutively in pepsin hydrochloride for 3 hours and alkaline pancreatin solution for 12 hours at 37°C. These enzyme treated drugs were then incorporated in the Löwenstein Jensen medium as described above. A few drugs were also treated with a drop of tween "80" before incorporating

TABLE I

| THE DRUGS TESTE | MAXIMUM EFFECTIVE DILUTION AGAINST M. TUBERCULOSIS H 37RV. | |
|---|---|---|
| | *Bactericidal* | *Bacteriostative.* |
| 1. p-Nitro-p'-aminodiphenyl Sulphone. | 1000 | 16000 |
| 2. p-Acetamido-p'-Nitrodiphenyl Sulphide. | 1,000 | 1,000 |
| 3. (a) D.D.S. | 16,000 | 64,000 |
| 3. (b) p-Nitro-p'-aminodiphenyl Sulphide. | 1,000 | 1,000 |
| 4. p-Tosylamino-p'-Nitrodiphenyl Sulphide. | 1,000 | 1,000 |
| 5. Gindarine Nitrate. | 1,000 | 4,000 |
| 6. Chaksine Iodine. | 1,000 | 1,000 |
| 7. Hyatin 1. | 1,000 | 1,000 |
| 8. Hyatin 2. | 1,000 | 1,000 |
| 9 Lupeal. | 1,000 | 4,000 |
| 10. p-Tosyl propylamino-p'-nitrodiphenyl Sulphide. | 1,000 | 16,000 |
| 11. p-Tosyl propylamino-p'-nitrodiphenyl Sulphone. | 1,000 | 16,000 |
| 12. p-Tosylpropylamino-p'-nitrodiphenyl Sulphone. | 1,000 | 4,000 |
| 13. p-Tosylpropylamino-p'-Nitrodiphenyl Sulphoxide | 1,000 | 4,000 |
| 14. Hyatin methiodide. | 1,000 | 4,000 |
| 15. p-Tosylethylamino-p'-Nitrodiphenyl Sulphide. | 1,000 | 4,000 |
| 16. p-Tosylethylamino-p'-Nitrodiphenyl Sulphone. | 1,000 | 1,000 |
| 17. p-ethylamino-p'-Nitrodiphenyl Sulphone. | 1,000 | 1,000 |
| 18. p-Ethylamino-p'-aminodiphenyl Sulphone. | 1,000 | 1,000 |
| 19. p-Tosylmethylamino-p'-Nitrodiphenyl Suphide. | 4,000 | 16,000 |
| 20. p-Tosylmethlamino-p'-Nitrodiphenyl Sulphone. | 1,000 | 1,000 |
| 21. p-Ethylamino-p'-galactosidamino diphenyl suphones | 1,000 | 1,000 |
| 22. p-Ethylamino-p'-amino diphenyl sulphones. | 1,000 | 1,000 |
| 23. p-propylamino-p-amino diphenyl sulphones. | 4,000 | 16,000 |
| 24. p-propylamino-p'-amino diphenyl sulphones. | 1,000 | 1,000 |
| 25. p-Laurylamino-p'-amino | 1,000 | 1,000 |
| 26. p-Sec. Butylamino-p-galatoridaimno-diphenyl sulphones. | 1,000 | 1,000 |
| 27. p-octylamino-p'-glactosidamino diphenyl sulphones. | 1,000 | 4.000 |
| 28. p-n-Amylamino-p'-galactosidamino diphenyl sulphones. | 1,000 | 4,000 |
| 29. p-Hexylamino-p'-galactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 30. p-Lauryl-amino-p'-galactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 31. p-Butylamino-p'-galactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 32. p-propylamino-p'-galactosidamino. | 1,000 | 1,000 |
| 33. p-Ethylamino-p'-glactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 34. p-Isoamylamino-p'-glactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 35. p-Isobutylamino-p'-glactosidamino diphenyl sulphones. | 1,000 | 1,000 |
| 36. Sulphetrone. | 1,000 | 1,000 |

TABLE II

*Effects of insoluble drugs (1 in 1000) against various bacteria.*

| *Name of organism* | *Number of drugs (See list of drugs in table I)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 9 | 12 | 18 | 19 | 21 | 23 | B.S. | Penicillin O. 1U/CC |
| *Pseudomonas. Pyocyaneus.* | + | BS | + | + | B.S. | B.S. | + | + | + |
| *Staphylococcus aureus.* | + | BS | + | + | B.S. | B.S. | + | + | — |
| *E. typhi.* | + | BS | + | + | + | + | + | B.S. | + |
| *Streptococcus haemolyticus.* | BS | | BS | BS | + | + | + | — | — |
| *Shigella dysenteriae shiga.* | + | BS | BS | BS | + | + | + | B.S. | + |
| *Vibrio cholerae ogawa* 60. | + | * | + | — | * | * | + | — | + |

N.B. +, growth ; —, no growth ; B.S., Bacteriostatic.

*Not done.

TABLE III

*Maximum bacteriostatic dilution of oils against bacteria.*

| Name of organism. | Nimbidol | Ethyl Nimbidolate | Essential oil mixture | Fluffo oil | Sulfanilamide. | Sulfadiazine. |
|---|---|---|---|---|---|---|
| *Staphytococcus aureus.* | 100 | <100 | 10,000 | 0 | <10,000 | 10,000 |
| *Streptococcus heamolyticus.* | 100 | <100 | 10,000 | 0 | <10,000 | 10,000 |
| *PS. pyocyaneus.* | 10 | 50 | 10,000 | 0 | <1,000 | 10,000 |

N.B.—less than Essential oil mixture for cholera. Fluffo oil is a bland vegetable oil mixture containing cotton seed oil and other oils.

them in the medium to see if it helped in a dispersion of the drug in the medium.

The different dilutions of the drug in the Löwenstein Jensen medium were then inspissated at 75°C for two hours on the first day and one hour each day for next two days. They were then inoculated with 14 days' culture of Mycobacterium tuberculosis H 37 RV. As far as possible equal quantities of inoculum were used in each tube.

*Testing of insoluble drugs against other bacteria.*—Ten milligrammes of the drug previously sterilized are thoroughly mixed with a drop of tween "80" in a sterilized pestle and mortar and subsequently emulsified with ten cubic centimetres of molten nutrient agar (containing 2 per cent agar). Five cubic centimetres are put in a sterilized tube. With the other five cubic centimetres of the material further dilutions in nutrient agar are carried out. The tubes are slanted in the cold for quick setting. Without tween "80" substantial amounts of the drugs invariably settle down at the bottom, while with tween "80" and nutrient broth the results were not as good as with tween "80" and nutrient agar. The eighteen hours' cultures of various bacteria to be tested are then inoculated on the surface of the slants and results noted after 24 hours incubation at 37°C.

*Testing of oils against various bacteria.*—One cubic centimetre of the sterilized oil in question is mixed with a drop of sterilized tween "80" and is thoroughly emulsified with nine cubic centimetres of one per cent gum tragacanth in nutrient broth, one cubic centimetre of this emulsion is mixed with nine cubic centimetres of one percent gum tragacanth broth and so on. A bland vegetable oil like olive oil or cotton seed oil is emulsified with tween "80" and one per cent gum tragacanth broth to serve as control. Instead of one per cent gum tragacanth solution, gum arabic two per cent solution may also be used but gum tragacanth is somewhat superior. A mixture of one percent gum tragacanth and two per cent gum acacia is inferior to one per cent gum tragacanth alone.

The emulsion may be inoculated with bacterial suspension prepared with normal saline but the mixing of the bacterial suspension with the emulsion becomes easier if a heavy loopful of bacteria is at first mixed with a drop of tween "80" while preparing the suspension. It is then incubated for twenty four hours at 37°C. Further sub-cultures are made from it to test for bactericidal effect of the drugs. Other insoluble drugs may be tested in a liquid medium in the same way.

## *Discussion*

From the above experiments which were repeated a large number of times over a period of one year, it is clear that Löwenstein Jensen medium containing egg and potato starch is a very suitable medium to keep uniformly dispersed highly insoluble drugs. Addition of tween "80" apparently did not help in any better dispersion in that medium. Enzymatic digestion of drugs consecutively with pepsin hydrochloride and pancreatin at an alkaline pH did not improve the bactericidal or bacteriostatic properties of the drugs in question. On the other hand treating the insoluble drugs with tween "80" prior to their being incorporated in nutrient agar kept them uniformly dispersed in the medium. In the case of oils treating them with tween "80" and subsequently emulsifying with one per cent gum tragacanth in nutrient broth made them satisfactorily stable. The tween "80" in quantities used has not been found to exert any bacteriostatic action.

## *Summary*

1. A method has been worked out for the testing of highly insoluble drugs against Mye. *tuberculosis* by incorporating them in Lowenstein Jensen medium.

2. It has been found possible to test satisfactorily highly insoluble drugs by treating them with tween "80" and then emulsifying with molten nutrient agar.

3. Oils can be tested for their bactericidal and bacteriostatic properties by treating with tween "80" and then emulsifying with one per cent gum tragacanth in nutrient broth.

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