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CHEMOTHERAPY OF TUBERCULOSIS

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INTRODUCTION

BEFORE the advent of modern chemotherapy, attempts have been made to cure tuberculosis with drugs without any success. The spectacular results obtained by Ehrlich with salvarsan aroused the optimism that this method could be extended to discover specifics for all infectious diseases. Thus, in the attempts to cure tuberculosis, innumerable compounds of diverse structures and groups, such as those derived from gold, silver, copper, mercury, cadmium and several other rare elements, calcium, arsenic, dyestuffs belonging to various groups, chaulmoogric acid and other related acid derivatives, etc., have been tried with disappointing results. The therapeutic effects obtained with even the best among them, *viz.*, the compounds of gold¹ do not stand comparison with those of Ehrlich. This lack of success with the bacterial infections as contrasted with the achievements with the protozoal infections such as trypanosomiasis and malaria, resulted in a sweeping rationalisation that chemotherapy cannot serve as a weapon for the conquest of the bacterial diseases.

However, the recent brilliant era in chemotherapy initiated by the discovery of prontosil, first dispelled the pessimism by providing spectacular cures against a number of deadly bacterial infections, the results obtained this time even eclipsing those of Ehrlich. The further developments in the subject from the practical and theoretical sides are now a common place. Researches on the wake of sulphanilamide forked out into two important directions. On the one hand, more and more powerful drugs were discovered, while on the other, the efficacy of these drugs in a large number of diseases was assessed. When it was recognised that the spectrum of antibacterial activity of the sulpha drugs was actually spreading out instead of the drugs remaining highly specific for just one infection, it was but natural to think of the sulpha drugs as suitable agents for destroying the tubercle bacilli and thus providing cures for tuberculosis.

SULPHANILAMIDE AND DERIVATIVES

Attempts at the synthesis and trial of derivatives of sulphanilamide as possible

cures for tuberculosis were started by 1938.² Rich and Follis³ reported that sulphanilamide, administered in quantities almost bordering on the toxic dose, was able to arrest the progress of tuberculosis induced in guinea pigs if the treatment was started simultaneously with the infection. Buttle and Parish⁴ found that sulphanilamide appeared to produce some degree of inhibition of an infection in guinea pigs with a human strain of tubercle bacillus; but the drug had little effect on the course of infection in guinea pigs and none in rabbits when a bovine strain was used. Sulphathiazole was found by Ballou, Guernon and Simon⁵ to exert bacteriostatic action on virulent human strains of tubercle bacilli on solid media and to arrest the development of experimental tuberculosis in guinea pigs. Following these reports, a number of investigators have tried many derivatives in experimental tuberculosis but the results are inconclusive; some⁶ have reported favourable effect if the drugs were administered in high doses, while others⁷ could not confirm this. Zucker, Pinner and Heyman⁸ tried sulphanilamide clinically on 13 patients giving the drug by the intravenous drip method maintaining a blood concentration of 17 to 32 mg. per cent. for 5 days at a time with no significant results. So far, no sulpha drug has established itself to be of any value in the management of tuberculosis.

LIPOPHILIC DERIVATIVES OF SULPHANILAMIDES

A number of fatty and other acid derivatives of sulpha drugs have been synthesised and tried on the basis of the heuristic hypothesis that the resistance of the tubercle bacilli to chemicals is possibly due to its protective fatty and waxy capsule and so any chemical with an inherent activity against the tubercle bacilli has a good chance in practice of killing the bacilli only if it could destroy or penetrate through the waxy capsule. This action on the waxy capsule is believed to be conferred on a compound possessing the fatty acid residue by virtue of its physical or "quasiphysical" properties.⁹ Thus, Bergman, *et al.*¹ prepared lipophilic naphthalene derivatives by condensing 4-benzencazo-1-naphthyl-

amine and 1-benzeneazo-2-naphthylamine respectively, with long chain acyl chlorides; in preliminary trials, some of them were definitely, though slightly, effective in experimental tuberculosis in guinea pigs and in experimental leprosy in Syrian hamsters. Bergman and Haskelberg¹¹ extended this work and have prepared a series of N⁴-acyl derivatives of sulphanilamide which have not been tested in experimental infections. Crossley, *et al.*¹² prepared a series of acyl derivatives of sulphanilamide; of these, N¹-dodecanoyl-sulphanilamide was reported to be very effective in experimental tuberculosis in guinea pigs.¹³ This claim has, however, not been confirmed by a number of other workers.¹⁴ Rajagopalan¹⁵ has synthesised a series of N⁴-acyl and N¹:N⁴-diacyl derivatives of a number of sulphanilamides of established value in other bacterial infections. Wagner-Jauregg¹⁶ prepared N⁴-chaulmoogrylsulphanilamide and has reported this compound to have no curative action either in leprosy in rats or tuberculosis in guinea pigs. The isomeric N¹-chaulmoogrylsulphanilamide is reported to be equal to sulphanilamide in efficacy in experimental streptococcal infections.¹⁷ Arnold¹⁸ has synthesised many 2-sulphanilamidothiadiazoles with higher alkyl substituents in position 5 of the thiadiazole ring; these compounds are easily lipid soluble but so far none appears to have been tried in tuberculosis or leprosy clinically.

SULPHONE DERIVATIVES

In the history of the chemotherapy of tuberculosis, it is only in 1940, that compounds (derivatives of 4-aminophenylsulphone) were found which were able to arrest definitely experimental tuberculosis in guinea pigs. Rist, Bloch and Hamon¹⁹ found 4:4'-diaminodiphenylsulphone to be far more effective than sulphanilamide against avian tubercle bacilli infections in rabbits. In a detailed study with this compound in experimental tuberculosis in guinea pigs lasting 8 months, Feldman, Hinshaw and Moses²⁰ found that 71% of the untreated controls died of tuberculosis, while only 29% of the treated animals died, part of even this mortality being due to the toxic effects of the drug administered. The high degree of toxicity of this compound made its clinical use as such undesirable. However, it was found that the protecting of

the amino group was a good method of masking the acute toxicity of the parent compound, at the same time making available a steady supply of the parent compound *in vivo* by hydrolysis. Of the many possible derivatives tried, three (promin, diasone and a phosphorylated compound) were found to be less toxic and of these the first two have been extensively tried in tuberculosis.

Promin (sodium 4:4'-diaminodiphenylsulphone-N:N'-didextrose sulphonate), though found to be far inferior to 4:4'-diaminodiphenylsulphone in its bacteriostatic action *in vitro*²⁰ for reasons explained above, has been found by Feldman, *et al.*²¹ to modify the course of infection and favour healing of tuberculosis in guinea pigs (*cf.* also Steekon, *et al.*²²). Promin has been tried clinically in pulmonary tuberculosis but it did not produce as good results as in the animal experiments. Diasone (sodium 4:4'-diaminodiphenylsulphone bisformaldehyde sulphoxylate) was found by Callowman²³ to be the most effective of the compounds tried in experimental tuberculosis in guinea pigs and also less toxic than the other sulphones. Feldman *et al.*²⁴ have confirmed these findings and produced more convincing evidence of the therapeutic value of diasone in experimental tuberculosis. As a result of this, a great deal was expected of diasone in the clinics. The controlled clinical trials with this drug have shown that this drug has not lived upto the expectations.

At this time, the heterocyclic derivative, promizole (4:2'-diaminophenyl-5'-thiazolylsulphone) was introduced as superior to diasone. Feldman, *et al.*²⁵ found this drug to be well tolerated and got very good results in experimental tuberculosis in guinea pigs. This drug was also tried by Feldman clinically. It is doubtful if it has been found to be better than the other sulphones. Just now, the interest in the sulphones was diverted to other channels.

Youman's and Doub²⁶ have tested the action of fifty-nine sulphone derivatives *in vitro* against virulent H 37 Rv strain of *M. tuberculosis*. These compounds comprise derivatives of 4-aminodiphenylsulphone and 4-aminophenylsulphone with a substituted heterocyclic ring. Only a few compounds came out showing activity comparable to 4:4'-diaminodiphenylsulphone. To check whether the *in vitro* method of testing is reliable as a short-

cut screening method to sieve out inactive compounds, Youmans, Feldman and Doub²⁷ have compared the effects of 33 compounds for activity *in vivo* and *in vitro*, the former tests being carried out in guinea pigs. The results have shown a fairly good correlation between the two methods so that we can take the *in vitro* tests as reliable for screening large number of compounds to have a qualitative idea of their activities.

Though the sulphones gave for the first time striking results in experimental tuberculosis in guinea pigs, the results obtained in the clinics are frankly disappointing. No significant advance was made in the practical side, though a fillip has been given to pursue the studies further.

STREPTOMYCIN AND THE ANTIBIOTICS

When the enthusiasm for the sulphones was dying down as a result of the clinical trials, streptomycin entered the scene with a great deal of partiality in its favour because of the spectacular performance of the other antibiotic, penicillin. Streptomycin gave such impressive results in experimental tuberculosis in guinea pigs²⁸ that it was hailed to be the long sought for specific for the conquest of tuberculosis. A number of reports on the action of streptomycin have been published, the most authoritative one being that of the Medical Research Council.²⁹ While no doubt streptomycin is probably the first drug to show very definite curative effect in tuberculosis, its use is limited. It has been found to be of value in acute and exudative cases of tuberculosis wherein the cavity has not developed, while it is of doubtful value in older cases. More extensive trials under controlled conditions will enable us to understand the merits and limitations of streptomycin therapy.

The puzzling feature about streptomycin, which greatly restricts its use, is the fact that *M. tuberculosis* exist (or develop?) which are not only resistant to streptomycin but also requiring streptomycin.³⁰ Those cases harbouring the organisms sensitive to streptomycin give good results by treatment for about two months. Where the resistant organisms predominate as a result of killing of the sensitive ones, the treatment with streptomycin only worsens the case. Since the percentage of the organisms resistant to or requiring streptomycin is only a small fraction, by combining streptomycin with another drug which can tackle the resistant and the "requiring" organisms,

we can hope to obtain very encouraging results. The value of streptomycin in tuberculosis can be appreciated only against the background of the previous consistently disappointing results obtained, in which state of hopelessness even the gold compounds were taken to be cures.

The example of streptomycin has led to the search for other antibiotics. So far none has entered the stage of clinical trials.

PARA-AMINOSALICYLIC ACID

When the mind is being prepared to accept as a dogma that the antibiotics are more likely to provide cures (and also give clues to synthetical investigations by suggesting possible structures), there is a swing back to the syntheticals by the discovery of the striking activity of such a simple compound as para-aminosalicylic acid. This compound was discovered by Lehman³¹ as a result of testing 60 compounds *in vitro* as a possible antagonist of salicylic acid which stimulates the respiration of the tubercle bacilli. The original claims of Lehman have been confirmed³² and this drug is being tried clinically. The past experience with clinical trials with compounds in tuberculosis urges a great deal of caution in giving out any pronouncement at this stage. But it is definite that the discovery of the therapeutic effect of this compound gives a very good lead to search systematically for possible cures even among compounds of simple structures.

FALLACIES ABOUT THE RESULTS IN ANIMAL EXPERIMENTS

A critical review of the literature of the chemotherapy of tuberculosis and particularly those published during the last ten years shows that the enthusiasm created by the animal experiments being damped by the actual clinical trials is a regular feature. There are many important reasons for this. In the first place, as far as the laboratory experiments are concerned, we did not have standardised techniques of animal experiments worked out yielding consistent results that could easily, without fallacy, be extrapolated to the human level. Secondly, the significance of the animal experiments as the index of their value in human tuberculosis in the various stages of the disease, was not fully appreciated and there was a hasty rush to report apparently favourable results. Lastly, the testing of the drugs clinically in such a chronic in-

fection—which presents diverse pathological pictures and the results being influenced by a number of factors ranging from psychological to nutritional is so time-consuming and tedious that it is difficult to realise and rectify the mistakes at an early date. This slows down the tempo of research also. Since it takes a long time to accumulate enough clinical data of significance to prove or disprove a claim, there is ample chance for any prejudice or favourite opinion to gain a strong foothold and misguide both the scientific workers and others. The case of the gold compounds is a good example for this.

In recent years, the methods of experimentation have improved. We are now convinced that a drug acts mainly on the parasite and it is axiomatic that the drug should act on the parasite *in vitro* if it is to be of value as a chemotherapeutic agent. Because of the slow rate of growth of the tubercle bacilli and that too in clumps, even the *in vitro* methods of testing of the activity of a compound as conducted previously did not yield reproducible results. The new methods now developed in which a new medium and the human strain can be used,³³ have vastly improved the position and is capable of yielding reproducible results in much shorter time. Thus, the initial screening method has speeded up the progress.

The animal experimentation technique using guinea pigs and the human strains of tubercle bacilli, is now well standardised. The method worked out by Dubos using white mice should indeed be a boon, if it is found to give reliable results. The results obtained with the sulphones in experimental tuberculosis is indeed suggestive of definite therapeutic action. But even in these cases, the results obtained correspondingly in the clinics are frankly disappointing. According to the principle of chemotherapy, the ideal conduct of the animal experiments should be such that animal infection should simulate the course of the disease in human beings as close as possible so that the results obtained in the former could be taken as an indication of what we can anticipate in the clinics. In many cases, particularly in the very acute infections such as those due to streptococci, pneumococci, meningococci, etc., the course of the infection in the infected animals (mice) and the human beings are not at all similar.

Still, the results of the animal experiments and those obtained clinically remarkably correspond in these cases; what appears to be of significance in deciding the clinical result is the fate of the parasite in its encounter with the drug in the system (of the animal or human beings). If a difference exists in the pathological picture of the experimental animal and the diseased human beings, or the drug undergoes different courses or degrees of absorption or metabolism in the two, then we should expect concrete difference between the animal experiments and the clinical results. In the case of tuberculosis in the guinea pigs, wherein the course of the disease is not as chronic and the pathological picture also not as complicated as that of the human being, the drug, if it possesses an inherent action on the tubercle bacilli, will have a good chance to encounter the bacilli and destroy them. Thus the drugs that show protective action in the experimental tuberculosis in guinea pigs no doubt indicate an inherent good action of the drug and satisfied an important requirement. The failure in the case of human tuberculosis, particularly in advanced cases, is probably because the drug has not been given a chance to encounter the bacilli; the devascularised character of the tubercular foci with the caseous area constituting the barrier zone between the circulating blood and the tubercle bacilli is responsible for this. So a drug to be useful clinically should also possess the property to approach and penetrate through the tubercle. Or else, the inherent tuberculostatic or tuberculocidal properties of the drug, however powerful it may be, will be of no use in actual practice. Such compounds can be used only in those cases wherein it could easily and directly be brought into contact with the bacilli, in such cases as tubercular meningitis, empyema, etc.

The pathological features of tuberculosis in human beings has thus presented chemotherapy with a very difficult problem. Since chemotherapy is concerned with the direct attack on the parasite, the only way it can solve the problem is by providing a drug which could eliminate completely from the system all the tubercle bacilli, whether free in the tissues or fortified within the devascularised foci, allowing the damaged areas freed from the parasite to recover with the aid of the body defence

forces. These conditions referring to the conduct of the drug in the system, extend the number of specifications of a good drug in addition to the inherent action on the bacteria. Since the drugs in the case of tuberculosis have to be administered over an extended period of time, the question of chronic toxicity is also of vital concern.

Only when all the above have been taken into consideration, one can appreciate why the advance in the case of tuberculosis is not as rapid as in the other acute diseases. The results so far obtained have given us definite leads enabling a rational attack of the problem. While we cannot possibly eliminate the sanatorium or the surgical treatments, we can hope to effectively supplement these established methods.

1. Amberson, McMohan and Pinner, *Amer. Rev. Tuberc.*, 1931, **24**, 401.
2. Ganapathi, *Curr. Sci.*, 1938, **6**, 608; *J. Indian Chem. Soc.*, 1938, 525.
3. Rich and Follis, *Bull. Johns Hop. Hosp.*, 1938, **62**, 77.
4. Buttle and Parish, *Brit. Med. J.*, 1938, **II**, 776.
5. Ballou, Guerson and Simon, *Amer. Rev. Tuberc.*, 1942, **45**, 217.
6. Feldman and Hinshaw, *Proc. Staff Meetings, Mayo Clin.*, 1939, **14**, 174; Karlsen and Feldman, *Proc. Soc. Exp. Biol. Med.*, 1940, **42**, 146; Follis, *Amer. Rev. Tuberc.*, 1940, **41**, 117; Birkhaug, *Brit. Med. J.*, 1939, **II**, 54; Smith, Emmart and Westphall, *J. Pharmacol.*, 1942, **74**, 163; —, *Amer. Rev. Tuberc.*, 1944, **50**, 163.
7. Kolmer, *et al.*, *Proc. Soc., Exp. Biol. Med.*, 1938, **39**, 581; Smithburn, *ibid.*, 1938, **38**, 574; Steinbach and Dillion, *ibid.*, 1939, **41**, 613; Heise and Steenken, *Amer. Rev. Tuberc.*, 1940, **42**, 801; Flippin, *ibid.*, 1940, **42**, 821; Callowman, *ibid.*, 1943, **47**, 97.
8. Zucker, Pinner and Heyman, *ibid.*, 1942, **45**, 292.
9. Robinson, R., *J. Chem. Soc.*, 1940, 506.
10. Bergmann and Haskelberg, *ibid.*, 1939, 1.
11. —, —, *J. Amer. Chem. Soc.*, 1941, **63**, 2243.
12. Crossley, *et al.*, *ibid.*, 1939, **61**, 2950.
13. Clemenko, *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 624.
14. Steinbach and Duca, *ibid.*, 1940, **45**, 556.
15. Rajagopalan, *Proc. Indian Acad. Sci.*, 1943, **18**, 108; 1944, **19**, 343.
16. Wagner-Jauregg, *Ber.*, 1942, **75**, 369.
17. Dewing, *et al.*, *J. Chem. Soc.*, 1942, 239.
18. Arnold, *Ber.*, 1942, **75**, 87.
19. Rist, Bloch and Hamon, *Ann. Inst. Pasteur*, 1940, **64**, 203.
20. Feldmann, Hinshaw and Moses, *Amer. J. Med. Sci.*, 1944, **207**, 290.
21. — *et al.*, *Proc. Staff Meetings, Mayo Clinic*, 1940, **15**, 695; *Amer. Rev. Tuberc.*, 1942, **16**, 197.
22. Steenken, *et al.*, *ibid.*, 1943; **48**, 453.
23. Callowman.
24. Feldman, Hinshaw, and Mann, *Proc. Staff Meetings, Mayo Clinic*, 1944, **19**, 25; Hinshaw and Feldman, *J. Amer. Med. Assoc.*, 1941, **117**, 1066; —, Pfeutze and Feldman, *Amer. Rev. Tuberc.*, 1943, **47**, 26; 1944, **50**, 52.
25. Feldman, Hinshaw and Mann, *Proc. Staff Meetings, Mayo Clinic*, 1940, **19**, 25.
26. Youmans and Doub, *Amer. Rev. Tuberc.*, 1946, **54**, 284.
27. —, Feldman and Doub, *ibid.*, 1946, **54**, 295.
28. Feldman and Hinshaw, *Proc. Staff Meetings Mayo, Clinic*, 1944, **19**, 593.
29. Feldman, Hinshaw and Mann, *Amer. Rev. Tuberc.*, 1945, **52**, 269.
30. Hinshaw, Feldman and Pfeutze, *J. Amer. Med. Assoc.*, 1946, **132**, 778; —, Pyle and Feldman, *Amer. J. Med. Sci.*, 1947, **II**, 429; *Brit. Med. J.*, 1943, **II**, 769.
31. Yegian and Vanderlinde, *J. Bart.*, 1948, **53**, 177; Splendon and Cummings, *Pub. Hlth. Rep.*, 1948, **63**, 1177.
32. Youmans, Raleigh and Youmans, *J. Bact.*, 1947, **51**, 409.
33. Dubos and Davis, *J. Exp. Med.*, 1946, **83**, 499; —, *et al.*, *Amer. Rev. Tuberc.*, 1946, **54**, 204.
34. Feldman and Hinshaw, *Amer. Rev. Tuberc.*, 1945, **51**, 582; Raleigh and Youmans, *J. Infect. Dis.*, 1948, **82**, 179, 205, 221.

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