# CHEMOTHERAPY OF TUBERCULOSIS

## RESEARCH DURING THE PAST 100 YEARS*

BY

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**1935–1946**

PART II

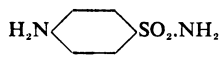
### The Sulphonamides and Sulphones

In 1932 Wells and Long suggested that the stimulus of some new success with other bacterial infections would be needed before the occurrence of any striking advance in the chemotherapy of tuberculosis. And in 1935, after 30 years of progress in the fields of protozoal and spirochaetal infection, but without the chemical mastery of any important general *bacterial* infection in humans, a substantial part of the bacterial front was broken. This, of course, was achieved by the successful use, in experimental streptococcal infection in mice, of azo-dyes containing a sulphonamide (—$SO_2NH_2$) group (Domagk). These were followed, first, by the dye-free sulphanilamide (*p*-aminobenzenesulphonamide, known since 1908), and then by numerous derivatives of the latter, known as the sulphonamides, and by various of the related sulphone (–$SO_2$–) compounds; and many of these synthetic drugs were introduced into medicine (Colebrook and others).

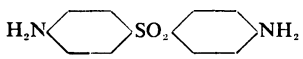
Encouraged by their reasonably high therapeutic index and their efficacy in acute infections, by their diffusibility, and by the presumption that their point of attack in the body was the parasites themselves, investigators tried a number of these sulpha drugs against tubercle bacilli in the test-tube and against experimental infection in animals, and a few of them in patients.

The first drug to be tested was the simplest—i.e., sulphanilamide (Rich and Follis, 1938; Follis and Rich, 1939); the net result of this and other inquiries was that in large and frequent doses some degree of inhibition of the disease in guinea-pigs could be obtained; but neither this compound, nor its derivatives sulphapyridine and sulphathiazole, nor others of the sulphonamide series, offered much hope of usefulness in the clinical sphere. A more promising field was opened up by the introduction, from 1939 onwards, of sulphone compounds into anti-tuberculosis chemotherapeutic experiments; the published data on these agents up to the end of 1944 have been admirably reviewed in detail by Tytler (1944–5), and only the salient aspects of the present position will be mentioned here. The structure of the more important sulphone compounds and, for comparison, that of sulphanilamide is shown in Fig. 2 (modified from Tytler), and it will be seen that three have two phenyl rings linked by the sulphone ($SO_2$) group, while the fourth compound is somewhat different, having a phenyl and a thiazole group linked by the sulphone group.

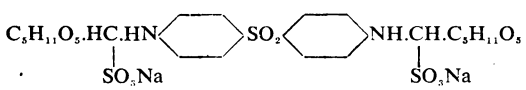
*Sulphanilamide* (=*para-aminobenzenesulphonamide*)



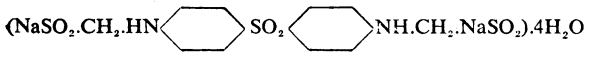
*Diaminodiphenylsulphone*



*Promin* (*Promanide*) (=*4, 4'-Diaminodiphenylsulphone-N, N'-dextrose sodium sulphonate*)



*Diazone* (=*Disodium-formaldehyde-sulphoxylate-diaminodiphenylsulphone*)



*Promizole* (=*4-Aminophenyl-2'-aminothiazolyl-5'-sulphone*)

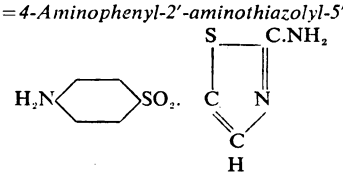


FIG. 2.—Structural formulae of certain sulphones.

The starting-point of the series, diaminodiphenylsulphone, showed some inhibitory effect in infected rabbits and guinea-pigs, but its low water-solubility and high toxicity were bars to its clinical application. The more complex "promin" (promanide) was easily soluble and had a low enough toxicity to warrant extended trials in experimental tuberculosis, which were given by Feldman and his colleagues at the Mayo Clinic and by other groups (see Tytler). The results showed this substance to be a striking inhibitor of the progress of the disease in guinea-pigs (the few observations made in mice—e.g., by Glover in 1945—were much less favourable). But when promin was tested in human pulmonary tuberculosis, although limited benefit was reported by some, the tolerance was found to be considerably lower than in the guinea-pig when the drug was given orally, unpleasant reactions—e.g., a (reversible) anaemia—occurring with some frequency; and while the toxic symptoms were much less evident after parenteral administration, so also was the clinical benefit (Zucker, Pinner, and Hyman, 1942; Heaf *et al.*, 1943; Hinshaw, Pfuetze, and Feldman, 1944; Dancey, Schmidt, and Wilkie, 1944). Experience with "diazone" in experimental tuberculosis in guinea-pigs resembled that with promin (see Tytler), but trials in human pulmonary disease produced conflicting reports, the majority of them unfavourable (Petter and Prenzlau, 1944; Benson and Goodman, 1945; Olson *et al.*, 1945; Pfuetze, 1945; Tice, Sweany, and Davison, 1946; Robitzek *et al.*, 1946). "Promizole" also gave encouraging results in experimental animals in the hands of Feldman, Hinshaw, and Mann (1944), who found it better tolerated by human beings than were the previously mentioned sulphone compounds. However, preliminary clinical trials of this compound in pulmonary tuberculosis have not indicated any marked superiority over promin (Hinshaw, Feldman, and Pfuetze, 1945); it is understood that a fully controlled clinical study is still in progress.

A fair summary of present experience with the sulphone compounds, as judged by those who have worked with them, is as follows. They exercise a deterrent effect on experimental tuberculosis, at least in some animal species, more than any previously tried chemotherapeutic agent; but, while early established lesions may regress and even be resolved, eradication of the virulent infection—a necessary criterion of cure in the acute or subacute disease of the hypersusceptible guinea-pig—is not attained. In man the small numbers and absence of simultaneous matched controls in most trials hitherto reported make assessment difficult, but the results appear nothing like as favourable as those in the guinea-pig; this is because a lower tolerance and risk of objectionable symptoms make adequate dosage difficult to achieve, or because the type of the disease is different, or possibly because the drugs may be altered in the body and become ineffective. At the best, some benefit in recent "exudative" lesions is attributable to these drugs, but there is not a consistent or unequivocal regression of the disease to quiescence or cure. Clinical use of those sulphones at present available seems likely to be limited to external or topical application,* to combination with other agents (e.g., streptomycin†), and to use in other mycobacterial infections (e.g., leprosy). "No definite place has been found for these drugs in treatment of the usual types of tuberculosis" (Hinshaw and Feldman, 1945a).‡

### Other Organic Compounds

Besides the sulpha drugs, a large number of organic chemical compounds have during the past decade been reported to be inhibitory to growth of tubercle bacilli *in vitro*, and a few of

* Conclusion of the Mitchell Lecture delivered before the Royal College of Physicians, London, on July 9, 1946. The first part appeared last week at page 805.

* Tytler and Lapp (1942); Lurie and Stokes (1943); Jarman and Morris (1945); Robin (1945); Grenville-Mathers *et al.* (1946).

† Smith and McClosky (1945a); Smith, McClosky, and Emmart (1946).

‡ For a recent evaluation of the efficacy of the sulphone compounds in experimental and clinical tuberculosis, see Feldman's (1946) Harben Lectures, which are quoted also in the leading article at page 862.

these to be active *in vivo*; a much larger number of *in vitro* positive results remain unpublished. A selected list of reported compounds is given in Table II, grouped according to chemical similarities. Some of them are purely synthetic artificially produced substances with no known counterpart in Nature; others are synthetic compounds related to some natural substance which has served as starting-point of the series; others are themselves of biological origin (these last are placed here rather than in Table III because they fall into convenient chemical groupings, whereas in Table III a provisional grouping by species of origin is adopted).

TABLE II.—*Organic Chemical Compounds Active against* M. tuberculosis in vitro, *or against Tuberculous Infection* in vivo, *grouped according to Chemical Similarities (Recent Publications)*

| Agent (and Reference) | *In vitro** | Experimental Animals† | Man† |
|---|---|---|---|
| *(a) Sulpha Drugs* | | | |
| Sulphanilamide[1] | + | + | − |
| Sulphapyridine[1] | + | ± | 0 |
| Sulphathiazole[1] | + | ± | 0 |
| Sulphadiazine[1 2] | + | ± | 0 |
| Further sulphonamides[3 33] | + | − or 0 | 0 |
| Diaminodiphenylsulphone[1] | + | + | 0 |
| Promin[1] | + | + | ± |
| Diazone[1] | ? | + | ± |
| Promizole[1] | ? | + | ? |
| Further sulphone-compounds[3 4] | + | + | 0 |
| *(b) Fatty Acids and Derivatives* | | | |
| Chaulmoogra derivatives[5 6 7 34] | + | ± | ? |
| Synthetic alicyclic acids[5] | + | ? | 0 |
| Branched-chain fatty acids and derivatives[6 8] | + | ? | 0 |
| Dialkyl succinic acid derivatives[9] | + | 0 | 0 |
| Unsaturated long-chain fatty acids[10 11] | + | 0 | 0 |
| Saturated long-chain fatty acids and esters[6 11 12] | + | + | ? |
| Fatty acid–dye compounds[13] | ? | ± | 0 |
| *(c) Aromatic Compounds* | | | |
| Benzophenone and allied compounds[14] | + | − | 0 |
| Naphthoquinone derivatives[15 28 32] | + | 0 | 0 |
| Naphthalene derivatives; amino- and nitro-compounds and aldehydes[32] | + | 0 | 0 |
| Iodobenzoic and iodosalicylic acids and other aromatic compounds[6 16] | + | + | 0 |
| *p*-aminosalicylic acid[17] | + | ? | ? |
| Halogenated phenyl ethers[34] | + | 0 | 0 |
| Thymol[18] | ? | + | ? |
| 4-*n*-alkylresorcinols[19] | + | 0 | 0 |
| Diethyl-stilboestrol[20] | + | 0 | 0 |
| *(d) Dyes* | | | |
| Acridine derivatives[21 22] | + | − or 0 | 0 |
| Indulin[23] | + | 0 | 0 |
| Various dyes[21 31] | + | − | 0 |
| *(e) Miscellaneous* | | | |
| Synthetic detergents[11] | + | 0 | 0 |
| Amido-compounds of α-furancarbonic acid[24] | + | + | 0 |
| 5-nitro-2-furaldehyde semicarbazone[25] | + | 0 | 0 |
| Urea[26] | + | 0 | 0 |
| Nicotinamide[27] | + | + | 0 |
| Calciferol[28] | 0 | 0 | + |
| Vitamin D and other hydrophenanthrene compounds[29] | + | + | 0 |
| 8-hydroxyquinoline sulphate[30] | + | 0 | 0 |

* Growth-inhibitory or bactericidal effect. † Systemic administration.
+ = Results with the listed substance (or with one or more of the substances in the listed group) reported positive. ± = Results slightly positive, inconclusive or conflicting. − = Results negative. ? = Results not known. 0 = Not tested.

*References.*—[1] See Tytler (1944–5) and text. [2] Smith and Oechsli (1945). [3] Sweaney, Sher, and Kloeck (1946). [4] Lehr and Bloch (1945); Smith and McClosky (1945b); Callomon and Raiziss (1946). [5] Emmart (1946). [6] Küster and Wagner-Jauregg (1944). [7] Prigge (1940, 1941); Buu-Hoï and Ratsimamanga (1942); Wagner-Jauregg (1943). [8] Robinson (1940); Wagner-Jauregg (1942); Buu-Hoï and Ratsimamanga (1944); Buu-Hoï (1945). [9] Barry and McNally (1945). [10] Bergström, Theorell, and Davide (1946). [11] Drea (1944). [12] Negré, Berthelot, Bretey, and Fethke (1945). [13] Bergmann *et al.* (1941). [14] Freedlander (1942, 1944); Feldman, Hinshaw, and Moses (1943). [15] Lloyd and Middlebrook (1944). [16] Saz *et al.* (1941, 1943). [17] Lehmann (1946). [18] McBurney, Cason, and Searcy (1945); Brooks (1946). [19] Drea (1946). [20] Faulkner (1944). [21] Cutting *et al.* (1945). [22] Avery and Ward (1945). [23] Kudryavtsev (1941). [24] Chpanir and Chertkowa (1944). [25] Dodd (1946). [26] Cummins (1942, 1945); but see Frisk (1946); [27] Chorine (1945); but see Paraf *et al.* (1945). [28] Charpy (1943–6); Dowling and Thomas (1945, 1946); Dowling (1946). [29] Raab (1946). [30] Courmont *et al.* (1936). [31] Willstaedt (1944). [32] Jouin and Buu-Hoï (1946). [33] Frisk (1946). [34] Burger *et al.* (1945). [35] Dubois (1942).

After the sulpha drugs, the most actively investigated compounds have been the chaulmoogra acids and structurally similar synthetic compounds, including synthetic cyclic and branched-chain, many-carboned fatty acids and their derivatives. Renewed interest in this group in tuberculosis springs partly from the historical favourable association of the chaulmoogra acids in the treatment of leprosy, and the earlier consequent research on tuberculosis with these and kindred synthetic products. But work in this field has received new encouragement from the chemical analyses by Anderson and co-workers, extending over the past 20 years, of the lipids of the mycobacteria (see Anderson, 1943). These analyses stimulated the synthesis, by other workers, of lipid-soluble or lipophilic substances that might be expected to penetrate the supposed waxy or fatty "envelope" of the bacillus, or to injure it—e.g., through a surface tension effect. Furthermore, since Anderson's researches revealed the presence in the tubercle bacillus of a series of new branched-chain saturated fatty acids, one of which—phthioic acid—produced tubercle-like local lesions in the guinea-pig, the experiments with various synthetic branched-chain fatty acids made some years before (see above) took on new significance; and the synthesis of fatty acids of similar type to phthioic acid, and so chemically related to a natural bacillary constituent, seemed to offer reasonable promise of blocking some essential biosynthesis (Küster and Wagner-Jauregg, 1944; see also Polgar and Robinson, 1945). Thus there are at least two ideas on the mechanism of growth inhibition—one physico-chemical (fat lysis or penetration) and the other chemical (synthesis-blocking)—guiding current work on the tuberculostatic action of fatty acids and their derivatives. Unfortunately, tests against tuberculosis *in vivo* with selected *in vitro* active substances of this group have so far been little less disappointing than was the earlier work. Moreover, the hypothesis that antibacterial properties can be related to structural similarities with essential metabolites has still to be proved applicable to *M. tuberculosis* (see later). Finally, the results obtained with water-soluble sulphone compounds and with streptomycin, and the fact that the mycobacteria, despite their fatty structure, are more susceptible to water-soluble antiseptics than to fat-soluble compounds, emphasizes that the "lipophilic" approach to the chemotherapy of tuberculosis is not the only one, nor may it be the most fruitful (see discussions by Lehr and Bloch, 1945; Dubos, 1945, pp. 293–4).

The reasons for investigating the aromatic group of compounds, shown in Table II, are diverse. Benzophenone and allied compounds were tested because of their relationship to the sulphones. Napthoquinone derivatives represent an attempt to interfere with the bacterial metabolism through a structural similarity to a hypothetical growth factor or growth stimulant of the vitamin K type. The use of iodobenzoic, iodosalicylic, and *p*-aminosalicylic acids arose from a search for growth inhibitors whose starting-point was the studies of Bernheim (1940, 1941) and others on the increase in oxygen uptake by washed suspensions of tubercle bacilli produced by benzoic and salicylic acids and certain other aromatic compounds; such studies suggested that these or chemically similar substances might play a part in the normal oxidations of the bacilli, and that substituted benzoates and salicylates might interfere with these oxidations and so inhibit growth. The recent renewed interest in dyes has its origin in the promise of the earlier work on tuberculosis by Lewis and others (see above); additional reasons for testing the acridine compounds (including atebrin) are their clinical value in protozoal and bacterial infections, while indulin was used because it was wax-soluble.

In none of this list of compounds has there as yet been strong evidence of efficacy combined with safety in human pulmonary tuberculosis, though with some of them the clinical trials are not completed.* In one non-pulmonary condition, however, a substance has given highly encouraging results in the clinic. The substance is calciferol, whose benefit in lupus vulgaris, when used in high dosage, has been reported by Charpy in France and by Dowling and Thomas in this country, working independently. The results would seem striking and consistent enough to convince without simultaneous controls, and they appear to exceed those obtained in this disease with any previous internally administered remedy. It is to be hoped that further work will confirm and extend these observations, and provide an explanation, e.g., as to whether the calciferol is supplying a deficiency or is acting as a chemotherapeutic agent in the stricter sense. Moreover, while serious toxic reactions have

* A recent Swiss report (Junod, 1946) summarizes work in Japan with the alkaloid cepheranthine (from *Stephania cepherantha* of the family of Menispermaceae). It is active *in vitro* against tubercle bacilli, has low toxicity and is stated to produce arrest, reversal, and often resolution of the lesions of experimental tuberculosis. Improvement is claimed with minute doses in many types of clinical tuberculosis.

not so far been reported as accompanying this treatment, the possibility of calcium deposition in tissues will have to be watched.

### An Antibiotic Age?

Soon after the impetus given to the search for chemotherapeutic agents in tuberculosis by the introduction of sulphonamide drugs in 1935, the bacterial front was further weakened and broken by the successful exploitation of antibiotic substances, none of which had hitherto found an undisputed place in medicine. In 1939 Dubos reported the extraction, from culture filtrates of a spore-forming soil bacillus, of the crude material tyrothricin, from which the crystalline polypeptides gramicidin and tyrocidin were derived; gramicidin was particularly interesting as being active *in vitro* and *in vivo* against certain Gram-positive bacterial species, but its toxicity restricted clinical use to topical application. In 1940–1 the almost ideal chemotherapeutic properties of penicillin—extremely high efficacy in systemic infections by susceptible micro-organisms, little antagonistic action by body constituents, and virtual lack of toxicity—were demonstrated experimentally at the Oxford School of Pathology. These two events stimulated widespread reactivation of this branch of microbiology, and antimicrobial agents were looked for in a multitude of living sources. In this search tuberculosis has figured prominently, particularly as penicillin was found not to inhibit the growth of tubercle bacilli in the test-tube or to retard significantly the development of the disease in experimental animals (Abraham *et al.*, 1941; Smith and Emmart, 1944).

TABLE III.—*Substances from Natural Sources Inhibitory* in vitro *to Growth of* M. tuberculosis, *grouped according to Species of Origin (Recent Publications)*

| Species | Name of Agent | References |
|---|---|---|
| (*a*) Fungi: | | |
| *A. fumigatus* .. | Fumigacin* | Waksman, Horning, and Spencer (1942); Schatz and Waksman (1944) |
| *A. fumigatus* .. | Helvolic acid* | Chain *et al.* (1943); Jennings (1945) |
| *A. fumigatus* .. | Aspergillin | Soltys (1944, 1946) |
| *A. fumigatus* .. | (Unnamed) | Asheshov and Strelitz (1945) |
| *A. flavus* .. .. | Aspergillic acid | Goth (1945) |
| *A. flavus* .. .. | (Unnamed) | Bush *et al.* (1945) |
| *A. clavatus* .. .. | Clavacin† | Waksman, Horning, and Spencer (1942); Schatz and Waksman (1944) |
| *A. ustus* .. .. | Ustin | Kuroya (1945); Hogeboom and Craig (1946); Doering *et al.* (1946) |
| *A. albus, niger*, etc. .. | (Unnamed) | Zorzoli (1940) |
| *A.* (unnamed) .. | Mycocidin | Gerber and Gross (1945, 1946) |
| *A.* (unnamed) .. | (Unnamed) | Kallos (1945) |
| *P.* (unnamed) .. | (Unnamed) | Miller and Rekate (1944) |
| *F. javanicum* .. | Javanicin | Arnstein, Cook, and Lacey (1946) |
| *Ch. cochlioides* .. | Chaetomin | Schatz and Waksman (1944); Waksman and Bugie (1944); Geiger, Conn, and Waksman (1944) |
| (*b*) Actinomycetes: | | |
| *S. griseus* .. .. | Streptomycin | Schatz and Waksman (1944); Schatz, Bugie, and Waksman (1944) |
| *S. lavandulae* .. | Streptothricin | Schatz and Waksman (1944); Waksman and Woodruff (1942); Woodruff and Foster (1944) |
| *S. antibioticus* .. | Actinomycin | Schatz and Waksman (1944); Waksman and Woodruff (1941); Waksman and Tishler (1942) |
| *Pr. gardneri* .. .. | Proactinomycin | Florey, Jennings, and Sanders (1945) |
| *Pr. cyaneus* .. .. | Litmocidin | Gause (1946); Brazhnikova (1946) |
| (*c*) Aerobic spore-forming bacteria: | | |
| *B. subtilis* .. .. | Subtilina | Fontes Magarao *et al.* (1944) |
| *B. subtilis* .. .. | Subtilin | Jansen and Hirschmann (1944); Salle and Jann (1945) |
| *B. subtilis* .. .. | Bacillin | Foster and Woodruff (1946) |
| *B. subtilis* .. .. | Eumycin | Johnson and Burdon (1946) |
| *B. licheniformis* .. | Licheniformin | Callow and Hart (1946) |
| (*d*) Non-sporing bacteria: | | |
| *B. larvae* .. .. | (Unnamed) | Holst (1945) |
| Milk streptococcus .. | (Unnamed) | Mattick and Hirsch (1944, 1945); Mattick (1946); cf. Oxford (1944) |
| (*e*) Higher plants: | | |
| *Allium sativum* (garlic) | (Unnamed) | Courmont *et al.* (1937) Rao *et al.* (1946) |
| Onion and garlic .. | Phytoncides | See Tokin (1945) |
| *Clitocybe gigantea* .. (fairy-ring mushroom) | Clitocybine | Hollande (1945) |
| *Plumbago europea* .. | Plumbagol | Saint Rat, Olivier, and Chouteau (1946) |
| Chlorophyll derivatives and related compounds | (Unnamed) | Daly, Heller, and Schneider (1939) |
| *Buellia canescens* (lichen) | Diploicin | Barry (1946) |

* Helvolic acid is related to, or identical with, fumigacin.
† Clavacin is identical with clavatin from the same species, claviformin from *P. claviforme*, and patulin from *P. patulinum*.
*A.* = *Aspergillus*. *P.* = *Penicillium*. *F.* = *Fusarium*. *Ch.* = *Chaetomium*. *S.* = *Streptomyces*. *Pr.* = *Proactinomyces*. *B.* = *Bacillus*.

In Table III are listed the majority of biological products recently reported as having tuberculostatic or tuberculocidal properties *in vitro*, under the headings of their microbial or other sources. It is already a formidable and rapidly growing collection; perhaps we are entering an antibiotic age in the chemotherapeutic investigation of tuberculosis. A notable feature of the list is the great preponderance of soil micro-organisms, showing the extent to which, in the past few years, soil and medical microbiology have become interconnected in a new way. It is evident also that there is particular concentration of interest around the genera *Aspergillus* and *Streptomyces*, and around the aerobic spore-bearing bacteria of the *B. subtilis* group. The range of activity of the antimicrobial substances produced by the various species listed extends in every instance beyond *M. tuberculosis*, though it is limited and selective; on the other hand, as with other antibiotics, the production tends to be species-specific or even strain-specific (as with streptomycin). In the great majority of the reports listed the *in vitro* evidence rests upon tests with culture fluids or crude concentrated extracts; in relatively few instances has a high degree of purification of the active substance yet been achieved. Names have, however, been conveniently created, even at an early stage of purification, to designate the active agent or agents. Some names—e.g., clytocybine, streptomycin, subtilin, and licheniformin—indicate the genus or species of origin; others are based partly or wholly on the action of the antibiotic—e.g., mycocidin.

In only a few instances among these biological products is there information upon a full trial of efficacy in experimental tuberculous infection, either because investigations have not yet reached this stage, or because the active substance has proved too toxic or is inactivated *in vivo*, or for other reasons. Up to date I am aware of complete reports of such experiments for only two substances—streptothricin and streptomycin.* Since the former showed undesirable toxic effects in laboratory animals and failed, in the dosage practicable, to control experimental tuberculosis in the guinea-pig (Feldman and Hinshaw, 1945a), it has dropped into the background.

### Streptomycin

Streptomycin was discovered in 1944 by Waksman, one of the world's leading authorities on the actinomycetes family, and his colleagues (Schatz, Bugie, and Waksman, 1944; for a review see Waksman and Schatz, 1945). This was the result of a purposeful search among soil micro-organisms for antibiotic agents active against the Gram-negative bacteria and the (Gram-positive) mycobacteria, and suitable for clinical application. The name streptomycin is "derived from the generic designation given to the sporulating and aerial-mycelium-producing group of actinomycetes—namely, *Streptomyces*" (Waksman and Schatz, 1945). The antibiotic was originally found in one particular strain of *Streptomyces griseus*, and, indeed, production has been noted in only a few of the other strains of this species since examined. Streptomycin has been obtained apparently pure. The low toxicity of the purified product for experimental animals (e.g., Molitor *et al.*, 1946; Molitor, 1946), its high power of inhibiting growth *in vitro* of *M. tuberculosis* (Schatz and Waksman, 1944; Emmart, 1945; Youmans, 1945; Youmans and Feldman, 1946), and its survival when introduced into the body, led to extensive tests in experimental tuberculous infection in guinea-pigs (Feldman and Hinshaw, 1944; Feldman, Hinshaw, and Mann, 1945; Smith and McClosky, 1945a) and in mice (Youmans and McCarter, 1945). In guinea-pigs the results of treatment begun simultaneously with, or some weeks after, infection were strikingly better than those with any previous chemotherapeutic agent, and the

* A recent note by Salle and Jann (1946) states that subtilin produces "a definite suppressive effect on the course of experimental tuberculosis in guinea-pigs."

substance was well tolerated. Not only was the development of the disease noticeably inhibited, but arrest and even eradication of established lesions were noted. In mice the effect was not quite so striking, possibly because of a different balance between the doses of drug and infection; definite suppression of the disease process was, however, evident. Owing to shortage of supplies, systematic clinical trials of streptomycin in tuberculosis have so far been mainly confined to its country of origin —the United States—under the supervision of the National Research Council (see Keefer, 1946), and the few published reports are of a preliminary nature (Hinshaw and Feldman, 1945b, 1946—54 miscellaneous cases with limited improvement in some; Cooke *et al.*, 1946—case of tuberculous meningitis with apparent recovery; Figi, Hinshaw, and Feldman, 1946—case of laryngeal tuberculosis with striking improvement; Cook *et al.*, 1946—12 cases of genito-urinary tuberculosis with improvement in some; Baggenstoss, Feldman, and Hinshaw, 1946—histopathology of regressing human lesions).

As seen from this side of the Atlantic, one might summarize the present status of streptomycin in tuberculosis as follows. This antibiotic seems more promising than any previous chemotherapeutic agent. In animals its toxicity is less than, and its effect on experimental infection better than, the best of the sulphone compounds. In man prolonged administration appears to have been free of serious and uncontrollable toxic reactions, though, as in animals, histamine-like responses, evidence of (reversible) renal "irritation," eighth-nerve symptoms, neuritis, and dermatitis have been reported; it seems possible that some of these reactions are due to impurities in certain of the preparations used. The effect on human tuberculosis justifies cautious optimism for certain forms of the disease, but, subject to any very recent information, Waksman's own words of November, 1945, still hold: "Prolonged treatment and studies of many cases are absolute prerequisites for any serious consideration of the efficacy of streptomycin in the treatment of tuberculosis. To date sufficient information has not yet been accumulated" (Waksman and Schatz, 1945). The need for caution has been learned from bitter experience of the past failures with gold, copper, and tuberculin, and from the false promise given by animal experiments with the sulphones. Should streptomycin occupy no more than a temporary place in tuberculosis therapy, the resulting increase in biological and chemical knowledge will nevertheless be very considerable and will assist in further advances.* †

Streptomycin has certain apparently unsatisfactory properties. Like penicillin, it is poorly absorbed from the alimentary canal, so that administration has to be parenteral. Unlike penicillin, it is a base and its activity *in vitro* is reduced by a lowered *p*H, which it might encounter in pus; how far this objection is of practical importance is not decided. A much greater possible disadvantage is that drug resistance is rather easily acquired by the tubercle bacillus and other susceptible micro-organisms, both *in vitro* and *in vivo* (Youmans and Feldman, 1946; Youmans *et al.*, 1946), and this is particularly liable to occur during a prolonged course of treatment. This is one reason why other chemotherapeutic agents would be desirable for use in tuberculosis, even should streptomycin justify present hopes.

### Outlook for the Future

The purpose of this historical review would not be served unless it helped in the assessment of the present position and future prospects. It is therefore relevant to consider some of the difficulties in the path of those who are searching for chemotherapeutic agents in tuberculosis.

In common with all chemical attack on systemic infective disease, the prime consideration is that the chemotherapeutic index of an agent must be reasonably high—i.e., that its effective dose must be substantially lower than the dose toxic to the host. This is where the great majority of prospective agents that are tuberculostatic in the test-tube fall down when tested in the infected body. Other general reasons are failure to disseminate so as to reach the infection site, inactivation by antagonistic substances in the blood or other body constituents, and excessive speed of destruction or excretion; while acquired resistance to the agent by the bacterial parasite is particularly liable to occur when, as in tuberculosis, prolonged administration is required. One general chemotherapeutic aim, however, may have to be modified in the case of human tuberculosis—namely, complete internal disinfection; in fact, when we consider the benign and possibly protective infection which so many of us harbour, the latter may not be necessarily even an essential objective.

There are also difficulties more peculiar to tuberculosis. To be directly inhibitory or lethal to this micro-organism an agent must penetrate the relatively avascular tubercle, enter the possibly vulnerable phagocyte that may enclose the bacillus, and penetrate the "hardy wax-armoured bacillus" itself (Wells and Long, 1932, p. 391); dense fibrotic encapsulation may also constitute a barrier. These difficulties led some workers to see more hope in the indirect attack—by chemical stimulation of the tissue cells around the tubercles, or of the host's defensive mechanisms generally, without contact with the bacilli, as is believed to be the mode of action of heavy metals, certain dyes, etc. Some of these obstacles have, however, probably been overrated. Thus Rich (1945) states: "The tubercle, in spite of its compact, solid appearance, must be permeable to substances in solution, else neither the cells nor the enclosed bacilli could receive the nutritive material necessary for their survival." Actually many diffusible foreign substances will pass from the blood stream into the centres of tubercles and caseous areas—e.g., various vital dyes, iodides, and iron—and some become concentrated therein; penetration of colloidal substances is, of course, much less likely, unless the molecules be small. Rich concludes that many chemotherapeutic substances can reach bacilli in epithelioid cells or in caseous lesions, but that when enclosed in living mononuclear phagocytes the agent must be capable of surviving the internal environment of such cells. If the agent reaches the bacillus, even the reputed waxy or fatty barrier, often supposed to be responsible for the remarkably low susceptibility of mycobacteria to many ordinary chemical antiseptics, seems doubtful in the light of the growth-inhibitory effect, both *in vivo* and *in vitro*, of water-soluble substances such as certain sulphones and streptomycin. Moreover, whether the abundance and variety of the lipids does or does not confer resistance, there is at present no proof that these lipids are organized as a cell capsule or envelope (see Dubos, 1945).

Special difficulties of a technical character also slow the rate of advance. On the biological side, *in vitro* tests are more time-consuming with *M. tuberculosis* than with many bacteria, since the organism grows slowly and uniform inocula and other conditions are not easily attained; recent improvements in media, in the use of submerged culture, etc., may assist (e.g., see Dubos and Davis, 1946). Animal tests also are prolonged, and they need larger amounts of the test substance than do tests in many other infections. The lesions produced in the commonly used experimental animals tend to be acute or subacute, and may differ in other respects from the chronic fibrocaseous lesions of reinfection comprising a large part of adult tuberculosis in man; and, although reinfection can be readily produced in animals, the difficulties of providing a uniform series for chemotherapeutic trials are increased. As to species of animal, the guinea-pig has been the animal of choice for most workers (Feldman and Hinshaw, 1945b), but owing to its lack of natural resistance and comparatively large drug requirements some have turned to the mouse, in spite of its rather atypical non-necrotic lesions (e.g., Glover, 1945; Youmans and McCarter, 1945; Martin, 1946). The chorio-allantoic membrane of the chick embryo is also used, but the tests are not yet on a very firm foundation and some of the techniques are open to criticism. Feldman and Hinshaw (1945b) have made excellent suggestions for standard procedures for chemotherapeutic tests in experimental tuberculosis, with special reference to guinea-pigs. Their criteria for a successful agent are

* For a recent evaluation of the efficacy of streptomycin in experimental tuberculosis, see Feldman (1946).

† A report of 1,000 patients with various infections, treated by streptomycin in the United States, has just appeared (National Research Council, 1946); 87 cases of tuberculosis of the more advanced types are included. Guidance on dosage and administration is given (see also Hinshaw and Herrell, 1946) and toxic manifestations detailed. It is concluded tentatively that streptomycin has a suppressive, though not usually eradicative, action in miliary, laryngeal, meningitic, skin, and renal tuberculosis, and in exudative pulmonary tuberculosis: there is still doubt as to the sequel after discontinuance of treatment. The side-reactions appear more common and somewhat more significant than earlier reports suggest, and certain of them are noted even with apparently pure preparations.

(abbreviated): (1) satisfactory tolerance and absence of serious or irreversible physiological derangements; (2) reversal of established progressive disease to non-progressiveness, resolution, fibrosis, or calcification; (3) eradication of virulent infection; (4) results to be achieved in a reasonable period. In such tests it would be desirable to use "strains of tubercle bacilli whose origin, type, and virulence are known and can, within limits, be guaranteed" (National Tuberculosis Association, 1946).

The preparation of synthetic substances and antibiotic material for *in vivo* trials against experimental tuberculosis requires much initial labour, making *in vitro* "screening" almost a necessity—even though the great majority of agents thus selected as worthy of further attention must be expected to prove ineffective therapeutically. Possibly some more significant screening tests than those that measure growth inhibition will be devised; present alternatives, such as observations on the effect of drugs on oxygen uptake of the bacilli in the resting state (e.g., Bernheim, 1941; Franke and Schillinger, 1944), are of doubtful value in this connexion. Prognostication of the effect in human beings from behaviour of chemotherapeutic agents in experimental tuberculosis provides further misleading possibilities. A good example of lack of correlation between results in test-tube, experimental animal, and man is given by the individual sulphonamides, the best of which are not highly active *in vitro* but give very encouraging results in guinea-pigs, yet have been disappointing clinically. However, few would oppose the principle that new agents of significant toxicity should be administered in human tuberculosis only after promising animal trials. This, of course, does not mean that efficacious agents may not still be first discovered at the clinic or the bedside—such success is shown strikingly in the recent treatment of lupus vulgaris with vitamin D.

The difficulties of evaluating chemotherapeutic trials in man are far greater than in experimental animals. Selection of cases provides a dilemma, for while improvement is most to be expected in early "exudative" lesions unaccompanied by much destruction or fibrosis (as revealed increasingly by mass radiography), these are just the cases that may improve with rest alone or no treatment at all; on the other hand, in chronic fibrocaseous disease the prospect of improvement is doubtful and, if it occurs, may be confused with naturally occurring spontaneous regression. Such considerations call for simultaneous matched controls, an adequate duration of the trial, and an intelligent use of statistics; however, owing to the very obvious obstacles in selecting controls and in giving them inert preparations for some months, most trials have relied instead upon the pre-chemotherapeutic behaviour of the treated patients and on the general clinical experience of the medical observers. Patients and controls should, so far as is possible, form homogeneous groups susceptible of classification, with emphasis in the first instance on bronchopneumonic, "labile," reversible changes. The foregoing difficulties and others inherent in clinical trials, as well as standard procedures to overcome them, are discussed by Hinshaw and Feldman (1944). There is divergence of opinion among experienced workers on some of the points; thus, Zucker, Pinner, and Hyman (1942) and Pinner (1944) hold that, with proper selection of material, preliminary results are obtainable in a brief period, and any positive effect should be obvious enough without controls. Now, if any type of case is suitable for non-controlled observation, it is the "acute" rapidly developing forms of pulmonary disease seen in young adults, and meningeal and some other non-pulmonary forms. One might therefore ask: Will a chemotherapeutic agent produce dramatic enough reversal of progression in such a case to prove that the agent was the determining factor, as has been achieved with penicillin in the absence of direct controls? It is a great deal to ask of it. Or will the ambiguities of "sanocrysin" be repeated? The current clinical trials of streptomycin are relevant to this question, and it should be noted that success has been reported in a few cases of tuberculous meningitis.

## Conclusion

Having thus discussed the history of, and some of the research problems particularly involved in, the search for chemotherapeutic agents in tuberculosis, we can see that two paths are being followed: by way of substances synthesized biologically by various forms of plant life, and by way of products synthesized in the laboratory by the chemist. Along the first path, as with the initial discovery of penicillin, little chemical principle is at present involved in the exploratory phases, which are therefore somewhat empirical. Along the second path the design of synthetic agents is being based on a number of suggestive "leads"; but one fundamental guide is as yet little developed. This is the hypothesis that many antibacterial agents function in virtue of structural similarities to substances that participate in the essential metabolic processes of the bacterial cells ("essential metabolites"), such chemical similarities leading to interference with these processes and so to inhibition of growth. This concept was much strengthened by the study in 1940 of the mutual antagonism between sulphanilamide and the closely related *p*-aminobenzoic acid in the inhibition of streptococci; and it has been used since as a basis for the design of fresh synthetic growth inhibitors for a variety of micro-organisms in which essential metabolites could be defined (e.g., see Knight, 1946). Knowledge of the metabolism of *M. tuberculosis* is, however, still very deficient, this micro-organism seeming able to use the simplest nutrients, to dispense with preformed growth factors, and to synthesize all its essential metabolites—at least after adaptation to the *in vitro* cultural environment in which it is usually studied. Efforts to define its essential metabolites, and to model growth inhibitors on their structure, have so far been confined to lipids, without as yet much result (see above); but further study of the nutritional requirements and metabolic reactions of this micro-organism as a whole may provide a valuable guide to the synthetic design of anti-metabolites that will block some biosynthesis essential to the functioning of its cells.

These two paths along which present work is moving, which originated separately about 50 years ago, can be expected to converge and ultimately to meet; for knowledge of the chemical structure of antibiotic substances may promote laboratory synthesis of active related compounds, while knowledge of the point of interference of such substances may give new information on the essential metabolism of the tubercle bacillus and lead to the purposeful creation of simpler synthetic anti-metabolites.

Should a real measure of success be reached with one or a number of chemotherapeutic agents this is not likely by itself to lead to the eradication of tuberculosis. During the past decade much else has been added to the understanding of this disease and to the ability to control it. Fuller appreciation of the importance of social factors in its incidence and spread, and of the value of social assistance (both financial and re-abling) in consolidating treatment; greater emphasis on the factor of household contact and on earlier diagnosis by means of mass radiography; and the coming to maturity of the various methods of collapse therapy and other surgical procedures: these are among the features of this period in the more favourably placed countries, such as Britain. It is improbable that success in chemotherapy will supersede all the tried and trusted methods of control acquired through the years which are applied to the individual patient, to his family, and to the community. Thus, rest can be expected to remain the foundation of treatment, and surgical methods to be needed in certain types of case; the conditions of life to which the patient returns will surely affect critically the ultimate results of even the most spectacular chemotherapy; and the state of housing and nutrition of the people generally may be expected to continue to influence the secular trend of tuberculosis mortality and incidence. There are in the world perhaps between 10 and 20 million sufferers from active tuberculosis. In order to reduce this inroad on world health we shall probably need most of the reasonable measures—social and economic, preventive and therapeutic—that we possess now or that we can acquire in the future. The attack will remain multiple; the tactics will change.

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# AMBIGUOUS INHIBITION OF STAPHYLOCOCCI BY PENICILLIN INACTIVATED WITH PENICILLINASE

BY

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For some time past penicillinase prepared by the growth of certain strains of *B. subtilis* in broth has been used in this laboratory to inactivate penicillin before testing for sterility. Each batch of penicillinase is assayed by a method devised in the Bacteriological Laboratory of Messrs. Boots Pure Drug Co., Ltd. (personal communication).

A 1:20 dilution of the penicillinase preparation in water is added in ascending quantities to a series of broth tubes each containing 4,000 units of penicillin. These are inoculated with a loopful of a broth culture of a sensitive staphylococcus, and the end-point is read after 40 hours' incubation at 37° C. On one occasion 20,000 units of penicillin was erroneously added to each tube in setting up the assay. On this being realized the penicillinase was diluted 1:4 instead of 1:20, the assumption being that this would give the same result, since the proportion of penicillinase to penicillin in each tube would be the same. However, no growth of staphylococcus occurred in any of the tubes. The assay was then repeated at the normal level of dilution and an end-point was obtained as usual near the middle of the range.

This incident suggested that the ability of staphylococci to grow in broth plus inactivated penicillin is limited to a certain range of concentration of penicillin.

### Experimental Results

It was first shown that failure to grow was not due to a change in *p*H of the broth. The following experiment was then carried out. Serial dilutions of penicillin in broth were made in geometrical progression, beginning with 200,000 units, the last tube containing 781 units. Corresponding dilutions of penicillinase in water were made and 1 ml. of each dilution was added to each corresponding tube of penicillin, so that the proportion of penicillinase to penicillin was the same in each tube. One loopful of staphylococcus broth culture was added to each tube and the series was incubated at 37° C. The tubes were examined first after 24 hours' incubation; growth had occurred only in the tube containing 6,250 units of penicillin. Examination after 40 hours showed growth in the tubes containing 12,500, 3,125, and 1,562 units, but no growth at the four highest dilutions or at the lowest one.

The experiment was then repeated using quantities of penicillin from 100,000 down to 390 units, and on this occasion examination of the tubes was made at hourly intervals. Growth occurred with 6,250 units at less than 19 hours (as it did in a control tube of plain broth), with 12,500 units in 22 hours, with 3,125 units in 24 hours, and with 1,562 units in 30 hours. No growth occurred with 100,000, 50,000, 25,000, 781, or 390 units up to 3 days.

The results of three further experiments are given in Table I.

TABLE I

| Penicillin (Units) | Expt. 1 | Expt. 2 | Expt. 3 |
|---|---|---|---|
| 100,000 .. .. | 0 | 0 | — |
| 50,000 .. .. | 0 | 0 | 0 |
| 25,000 .. .. | 0 | 0 | 0 |
| 12,500 .. .. | 0 | ± 3 days | (4) + 3 days |
| 6,250 .. .. | (2) + 22 hours | (3) + 26 hours | (1) + 21 hours |
| 3,125 .. .. | (1) + 21 ,, | (2) + 24 ,, | (2) + 23 ,, |
| 1,562 .. .. | (3) + 23 ,, | (1) + 22 ,, | (3) + 40 ,, |
| 781 .. .. | (4) + 30 ,, | (4) + 27 ,, | 0 |
| 390 .. .. | 0 | (5) + 40 ,, | 0 |
| 195 .. .. | — | ± 3 days | 0 |
| Control .. .. | + 18 hours | | |

0 = No growth. + = Normal growth. ± = Trace of growth. — = Not done.

It will be seen that growth always begins first in one of the tubes near the middle of the series and spreads outwards through the tubes on either side of the first, though the order is not constant. If the solutions in the high-concentration tubes in which no growth has occurred are diluted with broth to fall within the range of the positive tubes, growth immediately occurs, sometimes being visible in as short a time as three hours after dilution, showing that the penicillin has in fact been inactivated and that the staphylococci have not been killed by the high concentration, for only the original inoculum is still present. Similar results were obtained in other experiments, using three different samples of penicillinase prepared in media other than papain digest broth.

The penicillin used in all the foregoing experiments was manufactured some time ago and was relatively impure, containing 700 to 800 units per mg., assayed against staphylococcus.

Inhibition of growth is not due to anything in the penicillinase; this was shown by making serial dilutions of penicillinase in broth of the same strengths as used in the experiments with penicillin and inoculating with the same staphylococcus. Growth occurred in 18 hours in every tube. It seems evident, therefore, that there is some substance present with the penicillin which, while not affected by penicillinase, in high concentration inhibits the growth of staphylococci.

Confirmation of this hypothesis was obtained by repeating the experiment using purified penicillin, containing 1,600 units per mg., assayed against staphylococcus. An amount of this material containing 100,000 units was diluted serially and each tube was treated with the corresponding quantity of the penicillinase used in previous experiments. The tubes were inoculated from a broth culture of the same staphylococcus. Growth occurred simultaneously in 18 hours with the six highest concentrations, from 100,000 units down to and including 6,250 units. It occurred with 3,125 units in 21 hours, with 1,562 and 781 units in 40 hours, and with 390 units in 3 days. No growth occurred with 195 units up to 5 days. Inhibition at the low end of the scale thus occurs even with pure penicillin.

Three further experiments were done with penicillin from batches having potencies of 1,000, 1,210, and 1,450 units per mg. respectively. The results were as shown in Table II.

TABLE II

| Penicillin (Units) | 1,000 u./mg. | 1,210 u./mg. | 1,450 u./mg. |
|---|---|---|---|
| 100,000 .. .. | (7) ± 3 days | (5) — 3 days | (5) + 42 hours |
| 50,000 .. .. | (3) + 20 hours | (1) + 18 hours | (1) + 20 ,, |
| 25,000 .. .. | (2) + 18 ,, | (1) + 18 ,, | (3) + 24 ,, |
| 12,500 .. .. | (1) + 16 ,, | (2) + 20 hours | (2) + 22 ,, |
| 6,250 .. .. | (4) + 22 ,, | (3) + 24 ,, | (4) + 40 ,, |
| 3,125 .. .. | (5) + 40 ,, | (4) + 40 ,, | (6) + 3 days |
| 1,562 .. .. | (6) + 3 days | 0 | 0 |
| 781 .. .. | 0 | 0 | 0 |
| 390 .. .. | 0 | 0 | 0 |
| 195 .. .. | 0 | 0 | 0 |
| Control .. .. | + 16 hours | | |