REVIEW ARTICLE

THE CHEMOTHERAPY OF TUBERCULOSIS

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I. Introduction

It is difficult to understand, and perhaps easy to under-estimate the intractable nature of tuberculous infections, particularly in this age when almost all other forms of bacterial infection are amenable to chemotherapeutic control. Yet in many ways, infection by *Mycobacterium tuberculosis*, the causative organism of tuberculosis, is quite unparalleled by that of any other disease of bacterial origin. Several contributory factors may be cited, not the least of which are the characteristic slow growth of the organisms, their unique chemical make-up and the complexity of the lesions which are produced in the infected human subject. The formation and nature of the tubercle, the typical lesion of tuberculosis, and the bearing which this pathological structure has on the concentration of potential tuberculostatic agents in contact with the micro-organism itself have been fully described elsewhere[1,2] and it is not proposed to deal further with this aspect of the problem.

The vexed question of permeability of the bacterial cell itself is one with which all serious students of drug distribution mechanisms must continue to be concerned. It is realised that permeability of the tubercle does not necessarily imply permeability of the invading tubercle bacillus, although the importance of this factor can only be judged by comparison with other fields of chemotherapy. Indeed there is no direct evidence available to show that penetration of the tubercle bacillus is an essential prerequisite for tuberculostatic action. The early concept of a protective lipoid capsule[1,3] surrounding the organism has now been modified[4]. However, some tissue differentiation is implied by the isolation from the organism of two different polysaccharides, one associated with the lipoids and the other with the somatic portion of the cell[5]. Cytological studies with the electron microscope have revealed the presence of a thin cell wall and cytoplasmic membrane surrounding a central cytoplasmic mass[6]. Nevertheless, no satisfactory interpretation can yet be given of the selective action which the micro-organism is able to exercise over substances which are absorbed into the body of the cell. Whilst it must be admitted that the tubercle bacillus is undoubtedly resistant for long periods to relatively high concentrations of both mineral acids and bases[7], the argument which implies the failure of these substances to penetrate the cell tissues is not sound. This is implicit in the ready

absorption of other equally small molecules such as glycerol, asparagine, inorganic phosphates and magnesium salts, which together represent the organism's simplest nutritional requirements[8].

II. Biochemical Studies

In our present state of knowledge, the contributions which biological studies of *M. tuberculosis* can make to the chemotherapy of the subject are small, but of ever increasing importance. Such studies are informative about the mechanism of cell reactions and, for this reason, the following account of the various biologically active substances obtained from the tubercle bacillus has been included.

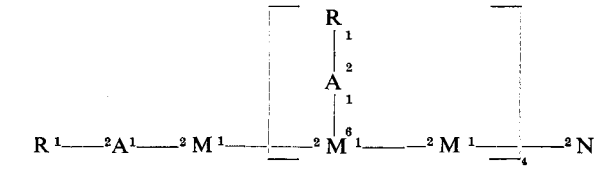
Carbohydrates

The presence of carbohydrates in *M. tuberculosis* was reported by some of the earliest investigators of its chemistry, but it was not until Laidlaw and Dudley[9] commenced their studies in 1925 that any real advance was made in the elucidation of their composition. They reported the isolation from dead bacilli of a specific polysaccharide, $[\alpha]_D^{20°C.} = +67°$, which on hydrolysis yielded a mixture of reducing sugars, including pentoses; it was highly reactive with the serum of animals immunised with dead tubercle bacilli. Later Maxim[10] and Heidelberger and Menzel[11,12] obtained similar dextrorotatory polysaccharides, of specific rotation $[\alpha]_D^{20°C.} = +72°$ and $+82°$ respectively; both these substances yielded D-mannose and D-arabinose on hydrolysis. Polysaccharides, which are undoubtedly identical with the above, have also been isolated from the bovine Bacillus Calmette-Guerin (B.C.G.) strain of *M. tuberculosis*[13] and also from various culture filtrates[14,15,16,17]. Heidelberger and Menzel[11,12] obtained evidence of a second specific polysaccharide of much lower dextrorotation, which also yielded D-mannose and D-arabinose on hydrolysis. This was confirmed by Renfrew's[18] isolation from culture media filtrates of a similar polysaccharide fraction, $[\alpha]_D^{20°C.} = +32°$.

More recent investigations[5] have confirmed the existence of two main polysaccharide fractions, one of high dextrorotation $[\alpha]_D^{20°C.} = +85°$ from the somatic portion of the organism and the other of low dextrorotation, $[\alpha]_D^{20°C.} = +25°$, associated with the lipoids.

The somatic polysaccharide, isolated by Haworth, Kent and Stacey[5], was obtained by alkali extraction, dialysis and alcohol fractionation. Repeated methylation yielded a brown gum free from all traces of desoxyribosenucleic acid impurities. Methanolic hydrochloric acid hydrolysis of this gum gave a mixture of methyl glycosides from which the following were fractionated and characterised:—3:5-dimethyl methyl-D-arabinoside, 2:3:4-trimethyl methyl-L-rhamnoside, 3:4:6-trimethyl methyl-D-mannopyranoside, 3:4-dimethyl methyl-D-mannopyranoside and an unidentified dimethyl methyl-2-aminohexoside, in the molecular proportions of 5:5:5:4:1 respectively. Of the many struc-

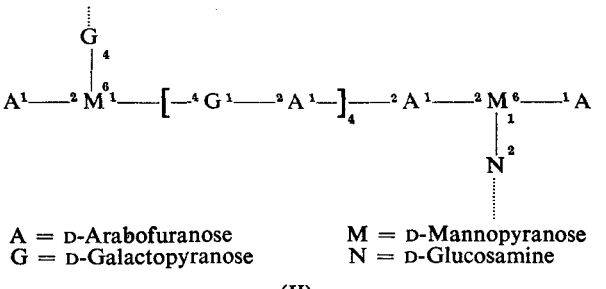
tures possible on the basis of this evidence, one suggested by Haworth, Stacey and Kent (I) is shown below:—



(I)

A minimum molecular weight of 12,000 from osmotic pressure measurements indicated that at least three such structures (I) must be joined together in the intact polysaccharide. End-group assay by the periodate method[19] showed the presence of only four rhamnose end groups to every twenty sugar units compared with five rhamnose end-groups estimated by methylation, suggesting that the three structures (I) are linked through their rhamnose units.

The *lipoid-bound* polysaccharide was isolated from a urea extract of heat-killed bacilli[5] and its chemistry was studied by a technique similar to that employed with the somatic polysaccharide. Methanolic hydrochloric acid hydrolysis of the fully methylated polysaccharide yielded 2:3:5-trimethyl methyl-D-arabinoside, 3:5-dimethyl methyl-D-arabinoside, 2:3:6-trimethyl methyl-D-galactoside, 3:4-dimethyl methyl-D-mannoside and a dimethyl methyl-D-glucosaminide, in the molecular proportions 2:5:5:2:1 respectively. II was suggested as a possible structure for the lipoid-bound polysaccharide.



(II)

Other polysaccharides have also been isolated from the tubercle bacillus. Anderson[20] reported the presence of inositol, mannose and an unidentified hexose in the acid hydrolysate of certain lipoid components of the tubercle bacillus and, more recently, Seibert[21] has described the isolation of two other polysaccharides by alcoholic fractionation of tuberculins, as yet, these fractions have not been identified with the two known polysaccharides.

All the various polysaccharide fractions, isolated from both the bacillus and from culture filtrates, are capable of reacting in precipitin tests with

serum from infected hosts; their action, however, is that of a hapten[9], since unlike the pneumococcus specific polysaccharides they are incapable of stimulating antigen formation. Neither do they induce hypersensitivity of the tissues.[22]

LIPOIDS

The isolation by Anderson[23] of the lipoids from *M. tuberculosis* and other acid-fast organisms and their separation into the three main fractions, phosphatide, acetone-soluble fat and wax has been reviewed elsewhere[2,24]. Several acids, specific to this group of micro-organisms, have been separated, of which the most important are tuberculostearic and phthioic acids, predominating in the phosphatide and acetone-soluble fat, and mycolic acid, the principal constituent of the wax fraction. Tuberculostearic acid is biologically inactive[24] and has been identified by Spielman[25] as (±)-10-methylstearic acid, a structure which has been confirmed by synthesis.

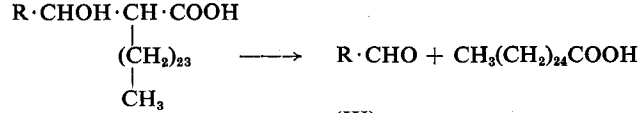
Anderson's phthioic acid, so named because of its capacity to elicit experimental tubercle formation, was originally isolated as an optically active white solid, to which the molecular formula $C_{26}H_{52}O_2$ was assigned. Alternative and more efficient fractionations by Polgar[26,27] and by Cason and Sumbrel[28] have now established that Anderson's phthioic acid was in fact a complex mixture; Cason and Sumbrel describe the separation of no less than eleven components from crude methyl phthioate[28]. The study of these acids has been further complicated by the use of a hydrogenation procedure in the earlier separations[29], based on the erroneous assumption that the zero iodine value obtained for crude phthoic acid implied the complete saturation of its constituent acids. Chanley and Polgar[27] have recently drawn attention to the fact that α-β unsaturated acids are unreactive toward halogens and have shown that certain phthioic type acids are, in fact, so unsaturated. One liquid acid, $C_{26}H_{50}O_2$, and two solid acids, $C_{28}H_{54}O_2$ and $C_{30}H_{58}O_2$ have been recognised and identified as α-β unsaturated acids by hydrogenation and by their ultra-violet and infra-red absorption spectre. Ozonolysis of the mixture of solid unsaturated acids, $C_{28}H_{54}O_2$ and $C_{30}H_{58}O_2$ and degradation of their hydrogenation products indicates the presence of a methyl group in the α position in at least one of these acids. Cason and Sumbrel[28] have provided additional information for the α-β unsaturation of the acid $C_{28}H_{54}O_2$ (C_{28}-phthienoic acid) isolated by fractional distillation of crude methyl phthioate. Kuhn-Roth estimation of terminal methyl groups established the present of at least three methyl branches to the aliphatic chain of C_{28}-phthienoic acid.

Earlier degradative and synthetic studies of Anderson's phthioic acid ($C_{26}H_{52}O_2$) have demonstrated that these substances are long-chain aliphatic acids. Stenhagen and Ställberg's formulation[30] as ethyl-*n*-decyl-*n*-dodecylacetic acid was shown to be untenable by Polgar and Robinson[31] and the alternative structure 2:12:18 trimethyltricosoic acid proposed on the basis of oxidation experiments[32] and compressed film measurements[33]. Synthesis of this acid and a number of other long-chain

aliphatic acids containing two, three and four methyl substituents indicated that acids of this type possessed biological properties similar to those of phthioic acid. However, in view of the evidence now available[27,28], it is not surprising that 2:12:18-trimethyltricosoic acid was not identical with the natural phthioic acid.

A series of di- and tri-substituted, saturated, long chain acids of the phthioic type has been synthesised, to determine the structural requirements essential to induce tubercle formation[34,35]. Tests conducted by Ungar, Coulthard and Dickenson[36] show that the C_{18} methyl substituent is not indispensable and that acids with but two methyl branches are only active when one is in close proximity to the carboxyl group. Unsaturation remote from the carboxyl group causes some decrease of activity; no α-β unsaturated acids were tested. A 1:1:dimethyltridecenoic acid has recently been shown to produce typical lesions[37]. Ungar[38] has shown since that particle size and surface area of the acid exposed to the tissues markedly effect the recorded activity. Speculating upon the possible function of the methyl substituents, Polgar, Robinson and Seijo[35] have suggested that the one nearest the carboxyl group protects the molecule against β-oxidation.

Mycolic acid from the wax fraction is now regarded as the substance responsible for the property of the tubercle bacillus known as acid-fastness[39]. Administered in the presence of tubercle protein, the wax fraction is capable of inducing a typical delayed hypersensitivity of the tissues, although neither wax nor protein alone show such effect[40]. Furthermore, the wax fraction is able to induce an analogous delayed hypersensitivity toward simple chemical antigens, such as picryl chloride[41]. Anderson assigned to mycolic acid the empirical formula $C_{88}H_{176}O_4$ and established the presence of hydroxyl, methoxyl and carboxyl groups in the ratio of 1:1:1. Pyrolysis yielded a mixture of products from which *n*-hexacosoic acid ($C_{26}H_{52}O_2$) was identified. Asselineau and Lederer[42] have now demonstrated the existence of the two isomers α- and β-mycolic acids and separated them by chromatography on alumina. The hydroxyl group in both isomers is established in the β position[43] in accord with the α-β unsaturated structures for the phthioic type acids, since dehydration gives anhydromycolic acid, itself an α-β unsaturated acid. The following mechanism has been proposed for the thermal splitting of both α and β mycolic acids to yield *n*-hexacosoic acid[44], analogous to the known splitting of β hydroxy-α-dialkyl-aliphatic acids[45,46].



(III)

The real course of the reaction seems to be more complicated since no aldehyde was isolated, but instead a mixture of methoxyl free substances, these as yet unidentified. The partial structure (III) has been

confirmed by dehydration to the corresponding unsaturated acid, anhydromycolic acid, and by its subsequent ozonolysis. The group R has been shown to contain one methoxyl and at least one side chain, since Kuhn-Roth estimation showed mycolic acid to contain three methyl branches[47].

PROTEINS

Studies of protein derivatives obtained from the tubercle bacillus have not yielded direct chemotherapeutic results, but they are of considerable importance from the immunological point of view. Rich[1] expressed the opinion that infection by the tubercle bacillus confers a mild degree of immunity against future attack upon the infected host. This suggestion has been largely substantiated by the use in more recent times of BCG vaccine, a living attenuated bovine strain[48] which confers a definite resistance in both laboratory animals and human subjects[49,50,51,52,53].

The active fraction of such vaccines appears to be extremely labile since no fraction, whether protein, carbohydrate or lipoid has so far been isolated from the tubercle bacillus which is capable of conferring immunity, though certain of the protein fractions are antigenic, producing a specific reaction in infected subjects. These same fractions are responsible for the delayed hypersensitivity and consequent necrosis of the surrounding tissue which accompany the development of the tubercle. In contrast, Rich and Lewis[54] and Maximow[55] have emphasised the lack of "toxicity" of multiplying virulent organisms for the non-sensitised tissue of an uninfected person. Such reactions can be stimulated not only by protein fractions isolated from the bacillus[56] but also with pure tuberculins[54,57,58] isolated from the medium in which the organisms have been grown.

The specific protein fraction, named tuberculin by Koch, is soluble in water and concentrates in the culture medium; it is this water-soluble fraction which has been so extensively studied. Seibert and her co-workers have isolated a number of highly purified protein derivatives and the results of this work have been admirably summarised in a number of reviews[58,59]. In particular, the isolation of a product, Purified Protein Derivative Tuberculin (P.P.D.T.), is described[59], special precautions being taken to prevent denaturation. Seibert[59] has demonstrated that this raw tuberculin contains at least two native proteins, designated A and B, which differ in solubility, coagulability, diffusability, sedimentability, electrophoretic mobility, antigenicity and potency. Both proteins are soluble at all hydrogen ion concentrations and have been separated on the basis of the lower electrophoretic mobility of the protein B. The protein B contains more polar groups, the dissociation constants of which suggest that they may be iminazole groups. Two partially denatured proteins A′ and B′ corresponding to the native proteins A and B have also been isolated by precipitation at *p*H 4·5. All four proteins exhibit tuberculin potency though this is less in the case of the denatured ones. More recent studies[21] have confirmed the existence of the two proteins A and B and indicated the presence of a third, C; evidence is presented by

McCarter and Bevilacqua[60] which suggests that the proteins A and B are quite unrelated and that there is no foundation for the suggestion that B is formed as a degradation product of A. The more refined technique of Heckley and Watson[61], in which the bacterial cell walls are ruptured by shaking with glass beads, has provided new evidence of substances more potent than P.P.D.T. in the water soluble extracts.

III. Chemical and Microbiological Studies

Streptomycins

Some thirty odd antibiotic substances, isolated within the last decade, are said to possess *in vitro* tuberculostatic properties[62]. Few, however, of these substances have yet been isolated in a pure condition and many of the antibacterial studies have been conducted with crude culture filtrates; only the streptomycins are used clinically.

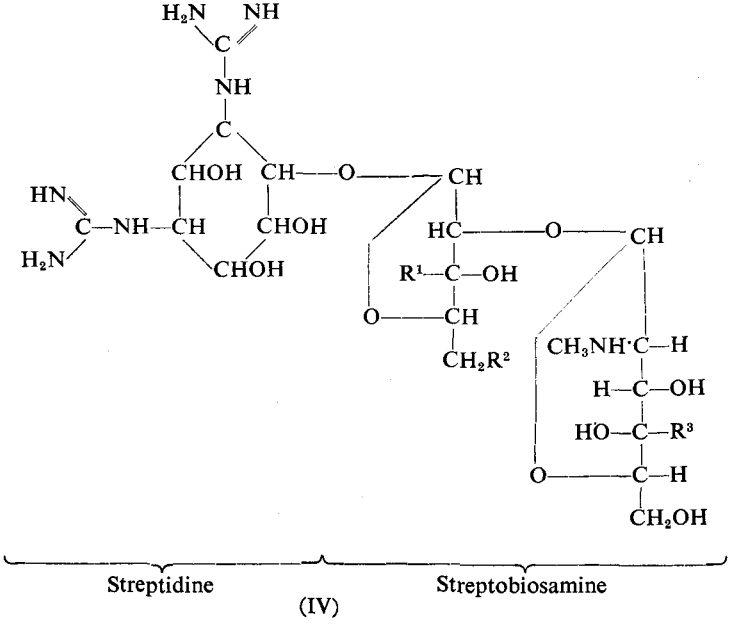
Streptomycin (IV R^1=CHO, R^2=H, R^3=H) was isolated by Schatz, Bugie and Waksman[63] as a metabolic product of *Streptomyces griseus*, which exhibited a high tuberculostatic activity *in vitro*[64,65] and in experimental animals[66]. Later reports of clinical trials with streptomycin were equally satisfactory, though its action appears to be limited to a suppression of the lesions rather than to complete irradication of the disease[67,68,69]. The vast number of reports published within the last few years has amply confirmed its early promise and there is no doubt that streptomycin is the most effective agent available for the treatment of tuberculous meningitis[70,71,72,73,74], pulmonary[75,76,77] and miliary tuberculosis[71]. Certain disadvantages attend its use. It is poorly absorbed from the intestines so that parenteral administration is essential. Moreover, the tubercle bacillus readily acquires resistance to streptomycin[78,79,80,81], making prolonged courses of treatment impossible, and even the purest forms of the drug give rise to neurotoxic symptoms[82,83]. Evidence is now available which suggests that combined therapy with streptomycin and *p*-aminosalicylic acid (P.A.S.) delays the onset of streptomycin resistance[84,85]. It is interesting to note that Fitzgerald and Bernheim[86] observed that streptomycin does not modify the increased oxygen uptake of pathogenic strains in the presence of benzoates and salicylates.

Dihydrostreptomycin (IV R^1=CH_2OH, R^2=H, R^3=H). The toxic symptoms of streptomycin appear to be due to the presence of the aldehyde (CHO) group, since dihydrostreptomycin[87] exhibits little evidence of toxicity[88]. *In vitro*[89] and *in vivo*[90,91,92,93] tests and also recent reports of clinical trials[87,94,95] all indicate that dihydrostreptomycin is just as effective against *M. tuberculosis* as streptomycin itself. This retention of activity by dihydrostreptomycin has been described as unique in view of the earlier observations that treatment of the aldehyde groups of streptomycin with carbonyl reagents, such as hydroxylamine and cysteine, render the molecule inactive[87]. It is now suggested that dihydrostreptomycin owes its activity to an *in vivo* oxidation to streptomycin.

Mannosidostreptomycin (IV R^1=CHO, R^2=H, R^3=D-mannose) was

isolated by Fried and Titus[96] by the application of Craig's counter-current distribution technique to a crude streptomycin concentrate. The product, streptomycin B, was renamed mannosidostreptomycin after its structure had been determined[97]. According to Rake *et al.*[98] it is about one-third as active as streptomycin both *in vitro* and in mouse tests.

Mode of action of streptomycin. This is still uncertain but considerable progress can be reported. Examination of the hydrolytic products has failed to reveal any particular fragment, which retains the full activity of the molecule as a whole. Acid hydrolysis yields streptidine[99] and streptobiosamine[100], neither of which has an inhibitory effect on the



(IV)

tubercle bacillus either *in vitro* or *in vivo*[99,101]. Attention has been directed to streptidine, which is a diguanidino derivative of *meso*-inositol, since the report that lipositol[102,103,104] an inositol-containing fragment of both brain and soya bean phosphatides, competitively inhibited the antibacterial action of streptomycin[105]. This evidence is now refuted by Paine and Lipman[106] who have shown that no antistreptomycin activity is exhibited by inositol containing phospholipoids. In spite of its apparent lack of antibacterial activity *in vitro*, there is no evidence to show that streptidine is not the active centre of the molecule. Indeed, it is tempting to suggest that streptobiosamine, through its *N*-methyl-L-glucosamine fragment, concentrates and, possibly, even orientates the streptidine fragment at the required site of action. In this respect it is significant to observe that, not only do both the somatic and lipoid-bound polysaccharides of the tubercle bacillus contain aminohexoses, possibly glucosamine itself, but also that the suggested formulation (I and II) for these polysaccharides[5] link an aminohexose directly to a D-mannose

unit in a manner which closely parallels the structure of mannosidostreptomycin.

A considerable body of evidence is now available which suggests that the action of streptomycin in both animal tissue and micro-organisms involves inhibition of carbohydrate metabolism, though the manner of inhibition seems to be specific to each individual case. In a recent report by Umbreit[107,108], the inhibition of fatty acid oxidation by streptomycin in *Bacterium coli* was traced to a block in the terminal respiration system at a point which involves a pyruvate-oxalacetate condensation. Streptomycin derivatives which show no antibiotic effect, including streptidine, did not affect this reaction[108] and furthermore streptomycin resistant strains were shown to have lost this condensation mechanism. Streptomycin inhibition of the pyruvate-oxalacetate condensation has also been demonstrated in tissue homogenates, though, in the intact animal, it appears that permeability factors prevent streptomycin from acting on the site of this reaction[109]. Henry, Housewright and Berkman[110] have also shown that streptomycin inhibits the utilisation of glucose and pyruvate by multiplying cells of *Shigella sonnei*. Similar impairment of carbohydrate metabolism is implied by the fall in the nitrogen and total phosphorus content of *B. cereus* and also by the accompanying increase of total reducing substances, which follows exposure to streptomycin. Evidence has been obtained that *Mycobacterium ranæ* oxidises pyruvate to acetate as part of its normal metabolism[111], and it is fairly general that oxidation of fatty acids is characteristic of mycobacteria[112,113]. Oginsky, Smith and Solotorovsky[114], in a study of fatty acid oxidation by avian strains of *M. tuberculosis*, have examined the possibility of streptomycin inhibition by a mechanism similar to the blocking of the pyruvate-oxalacetate condensation. Neither pyruvate, oxalacetate, mixtures of pyruvate and oxalacetate nor other members of the citric acid cycle were oxidised more than slightly, though some oxidation of higher fatty acids occurred by an unidentified mechanism, which was only partially inhibited by streptomycin. These results are in accord with the earlier observations of Bernheim[115], on the oxidation of fatty acids by the tubercle bacillus. Sym[116] reports a sixfold reduction in glycerol and amino acid consumption of *M. tuberculosis* in the presence of streptomycin and concludes that it is the assimilation, only, of such substrates as these which is affected.

Fresh lines of enquiry are suggested by the observations of Harper and Quastel[117] that the phosphorylation of D-glucosamine in acetone dried brain tissue is inhibited by *N*-acetylglucosamine and that this inhibition also extends to the normal phosphorylation mechanisms of fructose and glucose. The presence of the *N*-acetyl-L-glucosamine unit in the streptomycins calls for an early examination of the effect of these drugs on the phosphorylation mechanism of the tubercle bacillus. Several substances have been reported which antagonise the action of streptomycin, including urea, purines anl pyrimidines[118] and an antagonist of unknown

constitution produced by *Pseudomonas pyocyanea*[119], further investigation of which may provide useful results.

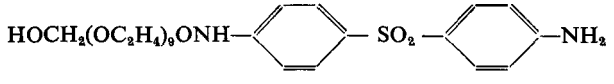
Hydroxystreptomycin (IV, R^1=CHO, R^2=OH, R^3=H). No evidence is yet available on the action of this antibiotic[120] against the tubercle bacillus.

Other Antibiotics

A number of antibiotics have been described, which are stated to possess activity against the tubercle bacillus; among others, these include licheniformin[121,122], nisin[123,124,125], neomycin[126,127], notatin[128], nebularine[129] and extracts from *Nocardia spp.*[130,131]. None of these substances appears to have progressed beyond the stage of examination in experimental animals. Penicillin, chloromycetin and aureomycin are without effect on the tubercle bacillus.

Long-Chain Aliphatic Acids and Bases

Although lipoid substances undoubtedly have an important role in the metabolism of the tubercle bacillus little success has so far attended the use of substances of this type as tuberculostatic agents. Both normal and branched chain fatty acids, including derivatives of chaulmoogric and hydnocarpic acids [132], have been examined and shown to exhibit *in vitro* toxity to the tubercle bacillus[133,134,135], but the results of *in vivo* tests have been disappointing. In contrast, Dubos and Davis[136,137] have shown that certain water-soluble esters of long-chain fatty acids, in particular oleic acid, favour growth of the bacillus. Eiseman[138] suggested that these substances, which are all surface active, concentrate at the periphery of the bacterial cell and made use of this property to increase the effective surface concentration of known tuberculostatic substances. Polyoxyethylene derivatives (V) of a number of sulphones were reported to show a thousandfold increase of activity *in vitro* over that of the parent



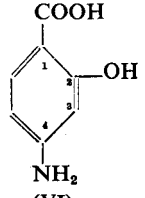
(V)

substance. Bailey and Cavallito[139] have similarly shown that combinations of streptomycin and aliphatic acids of chain length 10 to 18 carbon atoms are more active *in vitro* than streptomycin alone. Series of long-chain aliphatic diols and alkanolamines have been prepared by Barry *et al.*[140] for which high activity both *in vitro* and in experimental animals is claimed. Long-chain aliphatic diamidines[141] are also active *in vitro* but are too toxic for prolonged *in vivo* tests. Mixtures of long-chain aliphatic amines obtained directly by partial synthesis from natural phthioic acid were found to be active *in vitro*.

Para-Aminosalicylic Acid

The introduction of *p*-aminosalicylic acid (VI, PAS) for the treatment of tuberculosis arose from the observations of Bernheim[142,143] that the oxygen uptake of the tubercle bacillus was increased by benzoates and

salicylates. Lehman's further observation that benzoates and salicylates were only capable of influencing the oxygen metabolism of pathogenic strains of *M. tuberculosis*[144] and the subsequent examination of a large number of derivatives of these acids revealed that *p*-aminosalicylic acid[145] was tuberculostatic[146,147] *p*-Aminosalicylic acid has since proved to be effective in experimental animals[147,148] and in clinical trials on human subjects[149,150,151,152]. It is relatively non-toxic to humans[153] but, owing to its rapid absorption and excretion, frequent doses are necessary to maintain an adequate concentration in the blood. Combined therapy with sodium benzoate or caronamide retards the elimination and consequently increases the serum concentration of *p*-aminosalicyclic acid[154]. The influence of chemical constitution on the tuberculostatic activity of derivatives and analogues of this substance has been studied extensively[146,155,156,157,158,159,160]. The synthesis of a large number of compounds,



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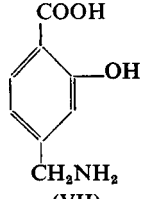


(VII)

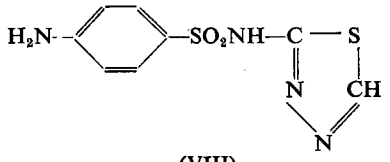
of which few equal the activity of *p*-aminosalicylic acid, has confirmed the original conclusions of Lehman:—(a) The OH group is essential in the 2 position. Replacement by NH_2 or Cl abolishes all activity, whilst removal to the 3 position causes a marked fall in activity. (b) Replacement of the 4-amino by a nitro or other grouping, or removal to the 3 or 5 position markedly decreases activity. (c) Alkylation of the amino group or esterification of the carboxyl group cause only slight reduction of activity.

Mode of action. No satisfactory interpretation is yet available of the above constitutional requirements. Some indication of a possible mechanism of action has been obtained from further studies of the respiration of the tubercle bacillus. Contrary to the observations of Lehman[144], Fitzgerald and Bernheim[161] have shown that several non-pathogenic strains of *M. tuberculosis* are capable of oxidising benzoates and salicylates. With pathogenic strains a similar increase oxygen consumption occurs in the presence of benzoates and salicylates, but there is no evidence that these substances are metabolised. It has therefore been suggested that acid-fast bacteria produce an adaptive enzyme in the presence of salicylates and it is this enzyme which is inhibited by streptomycin[161] and *p*-aminosalicylic acid[162]. This is substantiated by the fact that, although the tuberculostatic action of *p*-aminosalicylic acid can be reversed by sodium salicylate, some 4 to 12 mols. of the latter are required to inhibit the action of each mol. of *p*-aminosalicylic acid[162]. There is therefore no question of competitive inhibition of salicylic acid. A large number of compounds related to salicylic acid also failed to antagonise

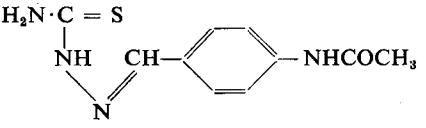
the action of *p*-aminosalicylic acid, but, on the other hand, both pantothenic acid[163] and *p*-aminobenzoic acid[156,157] act as antagonists. Carl and Marquardt[164] have recently proposed a generalised theory of action for certain tuberculostatics, including *p*-aminosalicylic acid, which involves the formation of a copper complex. This theory will be discussed later in connection with the thiosemicarbazones. 2-Hydroxy-4-aminomethyl benzoic acid (VII) the analogue of Marfanil has recently been reported[165]. Its action upon the respiration of avian strains of *M. tuberculosis* is similar to that of *p*-aminosalicylic acid, at first stimulating and afterwards inhibiting. Its tuberculostatic action is not reversed by *p*-aminobenzoic acid. The combined action of streptomycin and *p*-aminosalicyclic acid[166,167], which are synergistic, lends support to the idea that these two substances act by entirely different mechanisms.

THIOSEMICARBAZONES

Most sulphonamides are ineffective against the tubercle bacillus, but more definite activity is exhibited by those containing heterocyclic nuclei, such as sulphathiazole and sulphathiadiazole (VIII), from which the thiosemicarbazones are derived by structural analogy[168].



(VIII)



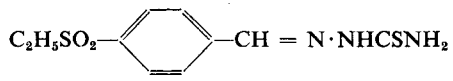
(IX)

4-Acetylaminobenzaldehyde thiosemicarbazone (IX, Conteben, Tb1/698), the most active thiosemicarbazone described by Domagk[168], has since been thoroughly examined. Early reports of its activity in mice showed it to be superior to *p*-aminosalicylic acid[169,170,171], but inferior to streptomycin[170,171]. Reports of large-scale clinical trials are now available, which support the earlier claims for its activity[172,173,174,175,176]*. Improvement is often rapid and marked, though toxic symptoms are frequently apparent[173,177] in the form of gastro-intestinal disturbances, anæmia[178] and severe liver and kidney damage[179].

Structural modifications of *p*-acetylaminobenzaldehyde thiosemicarbazone have already been examined[159,180,181,182,183]. The corresponding semicarbazones, oximes, hydrazones, azines and anils are inactive, showing that the sulphur atom exercises an important function in the determination of activity in this series[180]. Thiosemicarbazones of related aromatic ketones[168] and of heterocyclic aldehydes[182] show reduced activity; those from aliphatic carbonyl compounds are inactive[182]. Activity is increased by the presence of substituents in the aromatic ring, particularly those containing nitrogen, sulphur and oxygen.. Positional

* Domagk. Chemotherapie der Tuberculose mit der Thiosemikarbazonen, Georg Thieme Verlag, Stuttgart, 1950.

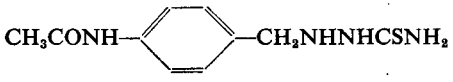
isomerism is also important, the general order of decreasing activity being *para* > *meta* > *ortho*. Of the *para* compounds, substituents rank in the following order, ethylsulphonyl = isopropyl > amino = acetylamino = dimethylamino > nitro = sulphamyl = methoxy[183], *p*-Ethylsulphonylbenzaldehyde thiosemicarbazone (X) has recently been described as



(X)

most promising[184,185] being much more active than the original *p*-acetylaminobenzaldehyde thiosemicarbazone.

Mode of action. Behnisch, Mietsch and Schmidt[180] have shown that the aldehydes are inactive, and also that thiosemicarbazide itself is only slightly active and very toxic. The inference that *p*-acetylaminobenzaldehyde thiosemicarbazone is not split in the body and owes its activity to the molecule as a whole is confirmed in the preparation of *p*-acetylaminobenzylthiosemicarbazide (XI). Hydrolytic cleavage is impossible,



(XI)

yet the compound is just as active as *p*-acetylaminobenzaldehyde thiosemicarbazone and in some respects is more advantageous because of its greater solubility and lower rate of excretion[186]. The addition of halogens and thiocyanogen to the azomethine link of the thiosemicarbazones similarly gives rise to stable and effective compounds.

A copper complex hypothesis. Carl and Marquardt[164], noting the chemical similarity between dithizone (phenylazothionoformic acid phenylhydrazide, XII)

$$C_6H_5N = N \cdot CS \cdot NHNHC_6H_5$$

(XII)

and *p*-acetylaminobenzaldehyde thiosemicarbazone (XI) and the ability of the former to co-ordinate copper, suggested that thiosemicarbazones would also form copper complexes. Such complexes are readily formed by thiosemicarbazones, *p*-aminosalicylic acid and a number of other tuberculostatic substances including diphenyl ethers, badional[175,176] (sulphathiourea) and dimethyldithiocarbamate[187,188]. It is suggested that a close parallel exists between tuberculostatic potency and the ability to co-ordinate with copper; but only in so far as this is implied by solubility of the complexes and in spite of very considerable variations of solubility from solvent to solvent. Support for the theory is claimed in the recent experiments of Schubert, Maurer and Riezler[189], who have shown, using radioactive copper, that this element accumulates to a much greater extent in tuberculous than in normal lung tissue. Moreover, anæmia resulting from the oral administration of *p*-acetylaminobenzaldehyde thiosemicarbazone has been shown to be due to a lowering of blood copper

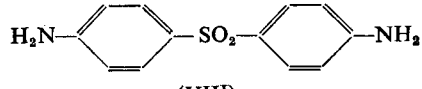
values[178]. On the other hand, it is difficult to understand why anæmias of this type do not appear to result from the administration of *p*-aminosalicylic acid, since the normal dosage ratios and copper complex solubility ratios of *p*-aminosalicylic acid to Tbl/698 are approximately of the same order. Further investigation particularly of stabilities rather than solubilities of such complexes appears to be justified.

Liebermeister[187,188] has described similar experiments with sulphathiourea, dimethyldithiocarbamate and Tbl/698, in which the pure preformed copper complexes were shown to exhibit tuberculostatic activities no greater than those of the original substances. Yet the activity of the pure thio substances is distinctly enhanced by even traces of copper sulphate; in contrast the fermentostatic activity of these substances is reversed by the addition of copper salts, and the copper complexes are ineffective as fermentostatics. It is clear, therefore, that the tuberculostatic action of these compounds, unlike their fermentostatic action, does not depend upon the withdrawal of copper from an essential enzyme system. Alternatively the complex, either preformed or formed *in situ* from traces of copper, must be considered to act as the specific poison. Schraufstätter[190] has examined a large group of substances for *in vitro* activity against *M. tuberculosis* and also for complex formation with copper, cobalt and zinc. According to his conclusions, however, there is no definite connection between complex formation and antibacterial action.

*iso*Thiosemicarbazones, *iso*thioureas and related substances whilst possessing appreciable *in vitro* activities, are inactive *in vivo*[191]. New heterocyclic derivatives of thiosemicarbazides are reported by Chalmer and Cattelain[192]; tuberculostatic activities are not discussed.

Sulphones

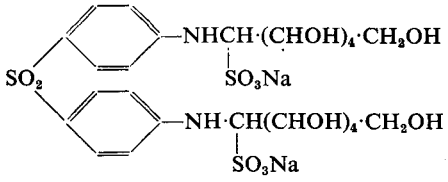
4:4′-Diaminodiphenylsulphone (XIII)[193], the parent substance of this group, was found by Rist to be active both *in vitro* and in experimental animals[195]. Its efficiency has been confirmed on many occasions, but all reports emphasise its very appreciable toxicity. Synthetic work has been directed as much to the reduction of its toxic reactions as to the provision of more active compounds. Lowe[196] has described the successful treatment of leprosy by oral administration of 4:4-diaminodiphenylsulphone



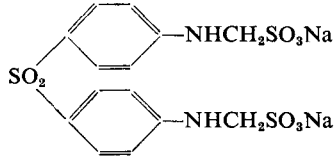
(XIII)

in graded doses over a period of several months, a form of treatment which slowly accustoms the patient to large doses previously considered too toxic. Large numbers of more soluble and less toxic derivatives have been prepared by substitution in the amino group; these include promin (disodium 4:4′-diaminodiphenyl sulphone *N*:*N*′-diglucose-

sulphonate, XIV)[197,198,199,200,201], diasone (4:4′-diaminodiphenyl sulphone disodium formaldehydesulphoxylate, XV)[202,203,204,205,206,207].

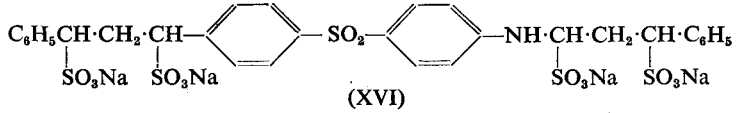


(XIV)



(XV)

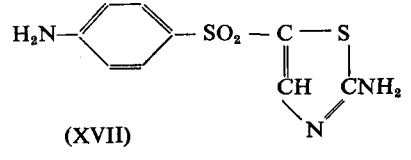
and sulphetrone (tetrasodium 4:4′-bis-γ-phenyl-*n*-propylaminodiphenyl sulphone tetrasulphonate, XVI)[208,209,210,211].



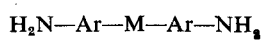
(XVI)

Evidence is available to show that derivatives of this type are degraded in the body to yield 4:4′-diaminodiphenyl sulphone and, presumably, it is to this re-formation of the parent compound that they owe their activity[212]. Numerous nuclear derivatives of 4:4′-diaminodiphenyl sulphone have also been examined[213,214,215,216,217,218] but, with few exceptions, they all exhibit only a low order of activity; certain hydroxy derivatives are much less toxic than the parent compound[217].

Heterocyclic sulphones[219,220] such as promizole (4:2′-diaminophenyl-5′-thiazolyl sulphone, XVII)[221,222,223], diphenyl and heterocyclic sulphides and sulphoxides, diphenyl ethers[224,225,226], diphenylamines[227] and diphenylmethanes[228] possess tuberculostatic activity. With the exception of promizole, few of these compounds have progressed beyond the stage of testing in experimental animals. Modifications of these substances giving rise to alternative but still analogous ring structures, such as phenoxanthins[218] and phenazines[227], have also been reported to show activity. Any substance of general formula XVIII, containing two suitably substituted aromatic ring structures (Ar) separated by a single group M, which may be —SO_2—, —SO—, —S—, —SO_2S, —NH—, —O— or —CH_2—, can be expected to show activity of the sulphone type. Little is known of the mode of action of these compounds beyond the fact that



(XVII)

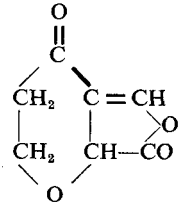


(XVIII)

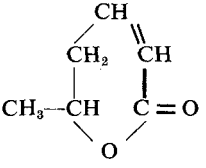
their activity, like that of *p*-aminosalicylic acid, is reversed by the action of *p*-aminobenzoic acid.[210]

α-β Unsaturated Carbonyl Compounds

Frahm and Lembke[229] have shown that α-β unsaturated carbonyl compounds, including patulin (XIX), parasorbic acid (XX)



(XIX)



(XX)

and benzoquinone inhibit the respiration of *M. tuberculosis*, probably through their action on the flavoprotein enzymes. Unfortunately, the effect is reversed in the presence of serum albumen, so that these substances are not practical agents for the treatment of tuberculosis. Thiosemicarbazones of aromatic aldehydes and ketones do not link with protein[230] and, although they do not effect respiration, this knowledge prompted an investigation of the thiosemicarbazones of α-β unsaturated carbonyl compounds. Both benzylidene-acetone and patulin thiosemicarbazones repress the *in vitro* growth of the tubercle bacillus to a high degree in the presence of serum.

These results may explain tuberculostatic studies with naphthoquinones related to vitamin K, which can also be regarded as α-β unsaturated ketones. The role of vitamin K in the metabolism of the tubercle bacillus is still uncertain, though a related pigment, phthiocol (3-hydroxy-2-methylnaphthoquinone) has been isolated from this source[231]. Several naphthoquinones tested as potential inhibitors showed high *in vitro* activity yet were quite inactive in experimental infections; this is now readily explained in terms of the serum inactivation of α-β unsaturated ketones. Other substances of analogous quinonoid structure such as the phenazines[227] and phenazine-*N*-oxides may also be found to owe their activity to a similar interruption of flavoprotein enzyme systems.

IV. Conclusion

This survey is not intended to be exhaustive, but sufficient has been said to indicate the use and limitation of the more important groups of compounds already established in the treatment of tuberculosis. At the same time, the hope is expressed that this account will stimulate interest in the microbiology of the tubercle bacillus. Knowledge of the cell reactions, as yet painfully inadequate, is fundamental to the rational advancement of chemotherapy in relation to tuberculosis and it is in this direction, more than any other, that the development of new methods for combating this micro-organism can most confidently be expected.

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