RECENT ADVANCES IN EXPERIMENTAL RESEARCH OF
TUBERCULOSIS CHEMOTHERAPY IN JAPAN:
A REVIEW

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The last decade was the most brilliant period in the history of anti-tuberculosis campaign since the time of Robert Koch. The appearance of some revolutionary chemotherapeutic agents has decreased the mortality due to this infectious disease in a definite way. This was particularly striking here in Japan where tuberculosis has been No. 1 killer for many years. Streptomycin was the first agent which evidently proved that tuberculosis can be cured by drugs. Ever since, being encouraged by this fact, further efforts have been concentrated on search for more effective and less toxic antibiotics or chemical compounds. The works have been conducted in a more extensive scale than the previous times, as they are called "screening test". Paraaminosalicylic acid (PAS) and isonicotinic acid hydrazide are the representatives among the drugs of signal value obtained in such an organized and systematic investigation.

Also in this country, works have been carried out in many laboratories searching for new antituberculous drugs. In fact, there have been presented some agents having definite activity against tubercle bacilli *in vitro* and sometimes even *in vivo*. Quite recently, after ten years of such kind of experience, this country has obtained her own antibiotic of clinical significance, kanamycin, as a powerful antituberculous agent equal in efficacy to streptomycin and isoniazid. Umezawa and his associates (1957) have the honor of the first discoverer of this antibiotic. They have been searching for basic and water-soluble antibiotics having no delayed toxicity, the idea being that such antibiotics would be active against tubercle bacilli equally *in vivo* and *in vitro* as preceded by streptomycin, fradiomycin and viomycin. Kanamycin was one of the products of such study. On the other hand, the author's laboratory has been in the position to be trusted with the test of newly isolated antibiotics and synthetic materials sent from many laboratories in this country for the presumed antituberculous activity. Those samples have been brought into our routine assay work one after another, mostly *in vitro* and sometimes in mice and in guinea pigs, to

examine whether or not they are truly active in inhibiting the growth of virulent tubercle bacilli. The works have been carried out for ten years without coming across any promising agents. However, we have finally had an opportunity to demonstrate that kanamycin isolated in our own institute is another potent antituberculous antibiotic. Of course, it must be noted that independent research works on tuberculosis chemotherapy have been also conducted in other various laboratories of this country, sometimes each standing on a different academic viewpoint and sometimes aiming at analysis of particular aspects of the problem.

At this time, the present author thinks that the time has come to review the recent works made by Japanese workers searching for antituberculous drugs and thereby to grasp the outlook on the future aspects of the problem. It goes without saying that the works in this country have been progressing under the influence of foreign literatures, and that this review should be written in the world-wide scope. However, it is beyond the author's capacity to make such a complete monograph of this problem in the limited space of paper. Therefore, the author should like to confine the scope within research works made in this country alone. If this review, although it may be quite sketchy, would be able to introduce the main aspects of the recent advances in experimental research of tuberculosis chemotherapy in Japan, the author's effort would be amply rewarded.

Antibiotics from Streptomyces

The history of systematic antibiotic research in Japan opened with the dramatic news of the discovery of penicillin in England during the war time. The Japan Penicillin Research Committee was organized inviting many workers in the fields of medical science, agriculture, botany and chemistry in February 1944. A cooperative investigation was made under difficult social conditions at that time, and the active element of penicillin was first purified in October 1944 by Umezawa and his associates (1948). Soon after the end of the war, the informations about streptomycin reached here to raise more interest of Japanese workers in this field of science, and the standard method of antibiotic isolation established by Waksman was introduced concomitantly. Although, for several years after 1945, the main effort of our antibiotic researchers was concentrated on the acquisition of the technique in industrial production of penicillin and streptomycin, the reports of new antibiotics have been increasing in number since 1949. Particularly, the greater part of their effort appears to have been paid on the discovery of antituberculous antibiotics. In fact, many of the antibiotics reported as tuberculostatic had no practical value, and some of them were identified with the antibiotics already discovered and defined in foreign countries. Nevertheless, the author should like to describe here the representatives of those antibiotics as the memorials left on the way leading to the kanamycin discovery.

Reticulin: Hosoya and his co-workers (1949) isolated a streptomycin-like

antibiotic from the culture liquid of a strain of streptomycetes, No. H-365 strain in their laboratory. This streptomycetes strain was assigned to *S. reticuli* and the antibiotic was named reticulin. Two years later Benedict and others (1951) also isolated a streptomycin-like antibiotic from a new species of streptomycetes named *S. griseocarneus* which is taxonomically different from the original streptomycin-producing strains, *S. griseus* and *S. bikiniensis*. This antibiotic was identified by them hydroxystreptomycin. Further two years later, Hosoya and others (1953) reported that although the producing strain is different in culture characteristics, reticulin is considered as hydroxystreptomycin. Miyamoto and others (1954) attempted a large scale of guinea pig experiment to test the comparative therapeutic activity of reticulin and streptomycin against experimental guinea pig tuberculosis. The result indicated that reticulin is active against experimental guinea pig tuberculosis in the same grade as streptomycin. However, cross resistance of bacteria was demonstrated concerning these two drugs.

A neomycin-like antibiotic: Hosoya and others (1949) isolated a streptomycetes resembling *S. fradiae* in culture characteristics. An antibiotic was isolated by them from the culture filtrate. Chemical analysis indicated that this antibiotic is composed of two sorts of unknown ninhydrin positive crystalline substances and glucosamine. When administered to experimental guinea pig tuberculosis with 1 mg daily dose, it inhibited the growth of tubercle bacilli in a remarkable way (Hosoya, 1950). However, no clear description was made concerning whether the antibiotic is identical with neomycin or not. Besides, any detailed data were not presented in their paper concerning the degree of toxicity in comparison with streptomycin and neomycin.

Thiazolidone antibiotic: This is a well-known example of the antibiotics which are not active *in vivo* despite of their strong *in vitro* activity against the growth of tubercle bacilli and of their extremely low toxicity. Maeda and others (1952) isolated an antibiotic of high activity to inhibit the growth of *Mycobacterium 607* from the culture liquid of a streptomycetes which had been obtained from a soil sample collected at Kanegasaki, Iwate Prefecture, as collection No. 154 T-3. Although the streptomycetes resembled *S. roseochromogenus* in some morphological and cultural characteristics, they defined the strain as a new species and named *S. cinnamonensis* n. sp. Test using a crystalline sample of the antibiotic indicated that it has a narrow antimicrobial spectrum to act only against mycobacteria and nocardia. The growth of these microorganisms was inhibited at dilutions of 1:160,000 to 1:2,560,000. LD₅₀ was larger than 400 mg per kg of mouse body weight when examined by intravenous injection. Just before their paper was pressed, however, two papers (Sobin, 1952) (McLamore *et al.*, 1952) had independently been published reporting a new streptomycetes antibiotic having the same chemical characteristics as their antibiotic. Maeda and others (1952) came to a conclusion that these two antibiotics are identical. American researchers had already proved the structure of this antibiotic as 2-(5-carboxypentyl)-4-thiazolidone and succeeded in its chemical synthesis. The observation

first pointed out by Sobin (1952) that this antibiotic is active *in vitro* but not *in vivo* was soon explained by Umezawa and others (1953), who demonstrated that the activity of the antibiotic is completely antagonized by biotin. On the other hand, acidomycin which was discovered and studied in detail by the researchers of the laboratory of Takeda Pharmaceutical Company Ltd. at that time was also identified as the same antibiotic as above (Hamada *et al.*, 1953).

Pyridomycin: This antibiotic was first isolated by Maeda and others (1953) from the culture filtrate of a new species of streptomyces obtained from a soil sample collected at Shirokane, Tokyo. Pyridomycin is a characteristic antibiotic having pyridin nucleus in its molecular configuration and a narrow antimicrobial spectrum. It acted to inhibit selectively the growth of mycobacteria such as *M. 607* and *M. phlei* at 1.6 μg per cc when tested by agar dilution method. Then, it was proved by Miyamoto (1955) that pyridomycin inhibits the growth of *M. tuberculosis var hominis* at 2~10 μg per cc in Kirchner liquid medium and is also active against streptomycin- and isoniazid-resistant variants in the same drug concentration. He proceeded to mice protection test with this agent. Pyridomycin was administered 8 mg daily for 23 days to mice intravenously infected with 1 mg of H37Rv tubercle bacilli. Survival effect was evaluated to be a little inferior to streptomycin (2 mg per day). Mice injected intraperitoneally with 30 mg pyridomycin in a single dose did not show any sign of toxicity. However, his further experiment (1956) using water-soluble pyridomycin hydrochloride failed to confirm his previous result. It did not act to suppress mice tuberculosis despite of increased administration dose and of various consideration of drug-administration route. Just at this time, Kuroya and his co-workers (1954) have discovered an antituberculous antibiotic from the culture filtrate of a streptomyces isolated in his laboratory as collection No. 6706. The antibiotic was specifically active against *E. coli* and *M. 607*. However, their chemical and biological analysis proved this antibiotic as pyridomycin itself. Although pyridomycin was not considered as an antibiotic of clinical significance, it has been studied in detail thereafter by Yagishita (1954, 1955, 1957) and Maeda (1957) from a purely academic interest of antibiotic research.

Phthiomycin: Phthiomycin is an antibiotic produced by *S. luteochromogenes Okami* isolated from a soil sample collected under bamboo-grass in Tokyo. The antibiotic was extracted from the culture liquid by the ion exchange resin process as the crude hydrochloride or the sulfate. Using a white amorphous sample of the antibiotic, the antimicrobial activity was surveyed. Like pyridomycin, phthiomycin showed a selective inhibitory activity against *M. phlei* at 6 μg per cc (Maeda *et al.*, 1953). LD₅₀ to mice by intravenous injection was about 600 mg per kg body weight. These observations led Miyamoto and Maeda (1954) to an attempt to examine whether or not phthiomycin is active against virulent tubercle bacilli *in vitro* and in mice. Mice intravenously infected with 1 mg of H37Rv tubercle bacilli were treated with phthiomycin in three different daily doses of 2 mg, 3 mg and 6 mg. For comparison, one group of mice treated with

2 mg of streptomycin and another group of non-treatment were arranged. Drug administration was initiated one day after infection. The result was that all the streptomycin-treated mice and those treated with 6 mg of phthiomycin survived during the observation period of 30 days, but those treated with 2 mg of phthiomycin survived in the rate of 11/15. The untreated animals survived in the rate of 5/15. At the same time, *in vitro* activity of phthiomycin was checked in Kirchner liquid medium against 14 different strains of mycobacteria including H37Rv and its variant substrains resistant to streptomycin and isoniazid. The test was made in comparison with the activity of viomycin which was considered to be similar to phthiomycin in chemical properties. In general, viomycin was superior to phthiomycin in the minimum inhibitory concentration in such a manner that the former inhibited the growth of H37Rv strain at 5 μ g per cc but the latter at 10 μ g per cc. The authors suggested that, as phthiomycin is less toxic than viomycin, the former would exert a more beneficial effect on mice tuberculosis when used in a larger amount of a more purified material. However, no further investigation was reported thereafter.

Grisamine: Grisamine is an antibiotic produced by a strain of streptomyces similar to *S. griseoflavus* (Sawazaki *et al.*, 1955). It was obtained in the form of needle crystals melted at 167°C to 170°C having $C_{20}N_4H_{30}O_7$ as its empirical formula. Grisamine inhibited the growth of BCG and virulent tubercle bacilli at the concentration of 5 μ g per cc in Kirchner liquid medium. Intravenous injection of 160 mg per kg caused the death of mice within one week, but 150 mg per kg was tolerated. Miyamoto and others (1955) conducted mice protection test against experimental tuberculosis with this antibiotic. Thirty mice of an inbred strain were infected with 0.001 mg of virulent tubercle bacilli (H2 strain). Grisamine administration to 10 of them was initiated one day after infection with 30 mg daily dose, and continued for 3 weeks. The effect was evaluated by viable bacterial unit count of the lung, liver and spleen, comparing with other two groups of 10 each. One group had been treated with streptomycin and another group had been left untreated. The result indicated that grisamine has no effect on mouse inoculation tuberculosis.

Homomycin: Homomycin was first isolated by Sumiki and his co-workers (1955) from the culture liquid of a new streptomyces named *S. noboritoensis*. This antibiotic was reported to have a high activity specifically against H37Rv tubercle bacilli and BCG. In addition, it was proved that homomycin has almost no acute toxicity, LD₅₀ being more than 2,000 mg per kg of mouse body weight by intravenous injection. These informations led Yanagisawa and others (1956) to an attempt to confirm the effect of homomycin *in vitro* and to extend the observation on mice experimental tuberculosis. The result was that homomycin inhibited the growth of H37Rv strain and its variant substrains resistant to streptomycin and isoniazid in Kirchner liquid medium at 1 μ g per cc or less than that, but it failed to prolong significantly survival time of heavily infected tuberculous mice. Nevertheless, these authors proceeded to a larger scale of guinea

pig experiment with a favourable expectation that it might be effective in this species of animal when used in an excessive amount. However, they soon encountered with a difficulty that homomycin exerted a delayed type of toxicity to guinea pigs after the administration for a week or so with over 50 mg daily dose, despite of its extremely low acute toxicity in a single injection. In this way, hope was abandoned of this antibiotic. Thereafter, Kawaguchi and others (1958) examined the effect of homomycin on experimental mouse leprosy, but the result was not favourable. More recent chemical and physical study of homomycin (Isono *et al.*, 1957), however, showed that this antibiotic is identical with hygromycin previously reported by Pittenger and others (1953) and Mann and others (1953).

Angustmycin: In the course of searching new antibiotics active against tubercle bacilli, Hsü Tüntsen and others (1954) isolated an antibiotic active specifically against *M. 607* and *M. phlei* but not active against H37Rv strain even at a concentration of 100 μ g per cc. Acute toxicity for mice was extremely low, being larger than 2.5 g per kg.

Orientomycin: Orientomycin was an undefined antibiotic produced by a strain of streptomyces isolated in Department of Bacteriology, Faculty of Medicine, Tohoku University. More recently, Shoji (1956) has studied in detail chemical and physical properties of this antibiotic and identified it with d-4-amino-3-isoxazolidone, namely oxamycin reported by Harris *et al.* (1955) or cycloserine reported by Harned *et al.* (1955).

Antibiotic 23572: Imamura and others (1956) reported a streptomycin-like antibiotic named tentatively Antibiotic 23572. The antibiotic was extracted from the culture liquid of a new species of streptomyces, *S. humidus nov. sp. Nakazawa et Shibata*. The *in vitro* and *in vivo* activity against tubercle bacilli was remarkable. However, their chemical and physical analysis identified this antibiotic with dihydrostreptomycin produced directly by the streptomyces. Tachibana and others (1958) confirmed that this antibiotic has the same antituberculous activity as dihydrostreptomycin currently in use in a guinea pig experiment.

Phleomycin: Maeda and others (1956) reported an promising antibiotic active against *M. phlei* and *M. 607* with little acute and delayed toxicity for mice. The strong activity against virulent tubercle bacilli was soon confirmed by Sato (1956). The phleomycin-producing streptomyces, collection No. 843-1, had been discovered by Utahara and others (1954) as an antifungal substance-producing strain. The recent study of Maeda and others (1956) showed that this strain produces three kinds of substances; the first is an antifungal one, the second inhibited the growth of *B. subtilis* although toxic for mice, and the third is phleomycin. Phleomycin is soluble in water and methanol but insoluble in other organic solvents. It has already been obtained in a crystalline form as a reineckate. While this is a promising agent, technical difficulties in industrial production is preventing further study of the activity against experimental tuberculosis.

Sinanomycin (Netropsin): Watanabe (1956) studied in detail an antibiotic named sinanomycin, a basic agent having a very broad antimicrobial spectrum including acid fast bacilli. This antibiotic had been obtained in his laboratory in a crystalline form from the culture filtrate of a strain of streptomyces, collection No. 7618, isolated from a soil sample collected near the Lake Noziri, Nagano Prefecture. His study identified this antibiotic with netropsin which had previously been reported by Finley and Sobin (1951). Despite of its high activity *in vitro*, sinanomycin has no possibility to be used as a drug because of its toxicity. LD₅₀ for mice was 10 mg per kg by intravenous injection.

Tubercidin: This antibiotic was obtained quite recently by Anzai and others (1957) from an undefined actinomyces strain isolated from the soil of Chiba Prefecture. Tubercidin is a basic and extremely stable crystalline compound decomposed at 247–248°C. Since LD₅₀ to mice by intravenous injection was reported about 45 mg per kg, the antibiotic would have no practical interest. However, according to them, tubercidin inhibited the growth of BCG organisms in Kirchner liquid medium at a concentration less than 1.0 µg per cc, although it has little or no activity against all other kinds of test microorganisms. Kanai and others (1957) proved that tubercidin can inhibit the growth of H37Rv tubercle bacilli in Kirchner medium at 3.2 µg per cc. One more interesting property of this antibiotic is that it has a strong anti-tumor action *in vitro* against NF mouse sarcoma.

Mycospocidin: This is an antibiotic reported by Nakamura and others (1957) as a product of a strain of streptomyces tentatively assigned to *S. bobilliae*. However, mycospocidin was differentiated from any other known antibiotics in chemical and physical characteristics. According to their test, *M. 607* and *M. phlei* were sensitive to this antibiotic each at 3 and 0.2 µg per cc. Spore bacteria were also sensitive in similar concentrations. LD₅₀ by intravenous injection was 1–2 mg per kg. Therefore, further study of the effect on virulent tubercle bacilli was not undertaken.

Elaiomycin: Quite recently Ohkuma and others (1957) extracted an oily antibiotic specifically active against *M. tuberculosis var. bovis* BCG from the culture broth of a strain of streptomyces similar to *S. hepaticus* excepting some morphological and cultural characteristics. However, the antibiotic was identified with elaiomycin previously reported by Ehrlich *et al.* (1954) as a new tuberculostatic antibiotic.

Aburamycin: Nishimura and others (1957) extracted a yellow crystalline antibiotic with weakly acidic properties from the culture broth of a new species of streptomyces, collection No. S-66, isolated from a soil sample collected from Aburabi, Shiga Prefecture. Chemical and physical properties of the antibiotic were studied in detail by them. The antimicrobial spectrum presented by them shows that aburamycin inhibits the growth of various species of bacteria including *M. phlei* but is not active against *M. 607*. LD₅₀ for mice was 2 mg per kg by intravenous injection. The effect on virulent tubercle bacilli was not reported.

Kanamycin: Umezawa and others (1957) considered from their experiences of antibiotic research that another powerful antituberculous antibiotic might be discovered among those basic, water-soluble and of no delayed toxicity. Streptomycin, neomycin and viomycin can be classed into this category. In fact, despite of the lipophilic nature of tubercle bacilli, antibiotics having non-polar and water-insoluble property are not active against them, especially *in vivo*. Kanamycin was a product of their systematic screening studies based on such an idea. A kanamycin-producing strain of streptomyces was isolated from a soil sample collected at Nagano Prefecture. Taxonomical studies on this streptomyces strain was made in detail by them to differentiate it from any other known organisms and assigned to a new species, *S. kanamyceticus* n. sp. Okami et Umezawa. Then, preliminary trials of the deep aerated tank fermentation were undertaken using the glucose meat peptone medium at pH 7.0. Antibiotic production was more abundant when pH rose to 8.0 than when it remained at 7.0 or so. The antibiotic was then extracted by the cation exchange resin process and crystallized as its reineckate. The hydrochloride sample gave positive ninhydrin and the specific rotation was $+103^\circ$, and it was differentiated from fradiomycins and catenulin by negative glucosamine test. Paperchromatographic analysis differentiated it from neamine. In addition, drug sensitivity test with *K. pneumoniae* and *B. subtilis* confirmed that the antibiotic is not identical with fradiomycins and neamine. The antibiotic was named kanamycin. Its antimicrobial spectrum was proved as broad as streptomycin. With much expectation, Takeuchi and others (1957) proceeded to the biological study of kanamycin. Their results are to be summarized as follows. Mice tolerated the single intravenous dose of 115 mg per kg, the single intraperitoneal dose of 544 mg per kg and the single subcutaneous dose of 563 mg per kg. Chronic toxicity was examined in mice and dogs administered with an over 100 mg per kg daily dose for 30 to 60 days. Change of body weight, liver and kidney function, and blood cell count were checked to prove that no toxic effect had been exerted on these animals. However, at the same time, it was found that the serum content of kanamycin decreased more rapidly than streptomycin to be excreted into urine. After these preliminary experiments, therapeutic effect of kanamycin on fatal pneumococcus and typhoid infection of mice was examined. The result was striking enough to encourage them to attempt further trials on various other experimental infections. At this time, a sample of kanamycin was trusted to the author's laboratory for the *in vitro* and *in vivo* test on virulent tubercle bacilli. Yanagisawa and Sato (1957) first examined its *in vitro* activity against various strains of tubercle bacilli and those resistant to streptomycin and paraaminosalicylic acid. The test was made in Kirchner liquid medium and Tween-albumin medium. The minimum inhibitory concentration was 5 μ g per cc in the former medium and 1 μ g per cc in the latter. This activity was a little inferior to streptomycin. Therefore, mice protection test was conducted in the following design. Twenty intravenously infected mice were divided into four groups of equal five; the

first untreated, the second treated with streptomycin of 1 mg daily, the third treated with kanamycin of 2 mg daily and the last with kanamycin of 4 mg daily. One day after infection, treatment was started and continued for 26 days. Then, all the animals were sacrificed and viable bacterial unit count was made concerning surviving tubercle bacilli in the lung, liver and spleen. The result indicated that kanamycin of 2 mg daily was as effective as streptomycin of 1 mg daily. This mice experiment was immediately followed by preliminary guinea pig experiments (Yanagisawa *et al.*, 1957). Although the experiments were conducted in a small scale, it was made clear that kanamycin of a 10 mg daily dose can prolong the survival time of heavily infected guinea pigs in a remarkable way, and that this beneficial effect was due to its activity to prevent the multiplication of tubercle bacilli *in vivo*. On the basis of these observations, a larger scale of guinea pig experiment was made using more moderately infected guinea pigs (Kanai and Yanagisawa, 1957). Kanamycin administration with a 10 mg daily dose was started two weeks after infection and continued for succeeding 9 weeks. The evaluation of efficacy was made in comparison with streptomycin and it was concluded that kanamycin has almost the same activity as streptomycin in experimental guinea pig tuberculosis. Additional experiments (Yanagisawa *et al.*, 1958) were attempted to examine kanamycin efficacy on experimental guinea pig infection with streptomycin-, isoniazid- and PAS-resistant tubercle bacilli. The effect was again striking proving that here is another potent antituberculous antibiotic. Clinical investigation of kanamycin on tuberculous patients is now under way in the selected hospitals and sanatoria, accumulating a number of successful cases. In the quite near future, kanamycin would be available in the world as one of the most effective drugs against tuberculosis.

Antibiotics from Lichens

As reviewed by Benedict and Langlykke (1947), antibiotic studies on lichens have been made by several authors indicating that this group produces some anti-tuberculous substances. In Japan, the antibacterial actions of lichen extracts were studied by Shibata *et al.* (1984). The chemical structure of more than 50 lichenic constituents, including fatty acids, depsides, depsidones, pigments of pulvinic acid derivatives, anthraquinones, phenanthrene-quinone, xanthine and diphenylene oxides, had been proved and some of them had the property of antibiotic. Particularly, usnic acid was found to have an *in vitro* bacteriostatic activity against avian tubercle bacilli in a high dilution. However, LD₅₀ for mice was 7.0 mg per 10 g by subcutaneous injection. The *in vitro* activity was decreased by blocking the chemically active groups, *i.e.* phenolic hydroxyl groups, doublebond, or cleaving the B ring of this acid. Usnic acid is a yellow crystalline pigment having a tricarboxylmethane group in its B ring. This fact led Ukita and others (1949) to a systematic study of antibacterial properties of the compounds having tricarboxylmethane group in their structure. More recently, Heki (1950) reported that usnic acid is active against experimental and human acti-

nomycosis. On the other hand, Kakimoto and others (1953) paid their attention to a coumarone group contained in the molecule of usnic acid. It is a fat and lipoid soluble group, possibly due to a phenylvinyl ether included in it. Starting from this consideration, they undertook the synthesis of several coumarone derivatives to test for their antibacterial activity against tubercle bacilli in Youmans' medium. However, significantly effective compounds were not obtained.

Antibiotics from Higher Plants

Cephalantin is an alkaloid contained in *Stephania cepharantha*, Hayata which grows wild in Formosa. Hasegawa first reported that this alkaloid has a remarkable inhibitory effect upon the formation of tubercles in experimental guinea pig tuberculosis. Until 1942, an extensive investigation had been conducted in this country to confirm and extend his observation. Clinical study was also attempted. In 1949, Hasegawa published a review summarizing those works. In fact, cephalantin appears to have some beneficial effects on skin tuberculosis and leprosy. However, according to Oka and Yanagisawa (unpublished data), this alkaloid has no inhibitory activity against the growth of *in vivo* tubercle bacilli, although it inhibits the formation of tubercles. Extending the work of Hasegawa, Nishikawa (1949) has made a screening test of nearly 2,000 plants growing in Kanto district of Japan for their antibacterial activity. However, no description was made regarding the effect on mycobacteria. More recently, Hasegawa and Tanaka (1952) found that cephalantin activates the popliteal lymphnodes of rabbits to increase the weight and the ribonucleic acid content, particularly eliciting the cellular response of plasmaoblasts and plasma cells. In addition, Tanaka (1952) showed the fact that cepharantin inhibits immune hemolysis. These observations appear to indicate that this alkaloid is an unspecific irritant to the cellular defense mechanism of animal body rather than an antibiotic specifically effective against tuberculosis.

Antibiotic from Bacteria

Uesaka and others (1955) have roughly investigated the antibiotic activity of 297 strains of mycobacteria and 52 strains of acid-fast nocardia. It was found that the antibiotic activity of these organisms were mainly against tubercle bacilli but not against *E. coli* or *M. pyogenes var. aureus*. Their antifungal activity was also not negligible. No growth of avian tubercle bacilli was observed in many cases on the plate when the active strains had been grown.

Synthetic Materials

Isonicotinic acid hydrazid derivatives: Isonicotinic acid hydrazid has the most strong activity to inhibit the growth of tubercle bacilli among several anti-tuberculous agents currently in use. However, efforts have been made by some authors to reduce its toxicity by coupling with other molecules and thereby to make it possible to administer a larger dose of the drug to human body. Kitamoto

and his associates (1953) prepared isonicotinyl hydrazid methansulfonate (IHMS) and proved that its subcutaneous LD₅₀ value for mice is 1106 mg per kg being 7 times less toxic than isoniazid (INH) itself. The *in vitro* antituberculous activity of IHMS was about the same as INH against virulent tubercle bacilli, although cross resistance of bacteria was demonstrated between these two drugs. Nishimura and others (1953) examined ketone derivatives of INH with the result that aceton-, methylethylketone-, acetophenon- and cyclohexanone-derivatives were more effective than INH. 2-Amino-5-alkylthiodiazol derivatives were inactive against H37Rv tubercle bacilli in Kirchner liquid medium. They (Nishimura *et al.*, 1954) further attempted mice protection tests against tuberculous infection using isonicotinyl-o-oxybenzal hydrazine or its meta- and para-isomers. The results were that the minimum effective dose of these INH derivatives was a little larger than INH itself but their tolerable dose was much higher than INH. Uchida and others (1954) also tested many kinds of INH derivatives without coming across any superior agents to INH. For instance, the minimum inhibitory concentration against tubercle bacilli was 0.5 μ g per cc in the case of lactose-isonicotinylhydrazone, 1 μ g per cc in N-oxide of INH, and 10 μ g per cc in 1-isonicotinyl-3-thiosemicarbazid. Kakimoto and Yamamoto (1955) prepared a salt-form compound between INH and PAS, and confirmed Brodhage's observation (Brodhage, 1954) that its activity against tubercle bacilli is stronger than a mere mixture of PAS and INH.

o-Aminophenol and related compounds: During his systematic research of tuberculosis chemotherapy since 1941, Okamoto (1948) found that *o*-aminophenol possesses the specific bacteriostatic activity against tubercle bacilli enough to inhibit their growth in dilutions of 1:256,000 to 1:4,000,000. This activity was specific and other species of bacteria such as pneumococci, streptococci, coli bacilli *etc.* were not inhibited. Its para- and meta-isomers were devoid of these activity. A 5% water solution of *o*-aminophenol with dextrin as the colloidal vehicle was prepared and applied to tuberculous patients. Some beneficial effects were observed without accompanying any unpleasant side reaction. Later, the anti-tuberculous activity of *o*-aminophenol was compared with streptomycin (Sagara *et al.*, 1949) and PAS (Sagara *et al.*, 1949). Miyamoto (1951) undertook a larger scale of guinea pig experiment to examine the antituberculous activity of the *o*-aminophenol preparation, but the result was not so favourable as expected from the above reports. Matsuda (1954) made an *in vitro* experiment to examine the emergence of *o*-aminophenol resistant tubercle bacilli, but the result appears to indicate that tubercle bacilli may not acquire resistance to *o*-aminophenol. Yoshimura *et al.* (1954) was stimulated by these *o*-aminophenol experiments and led to an attempt to test 3-(β -N, N-diethylamino-propionylamino)-phenoxazone-(2) for its presumed antituberculous activity *in vitro* and *in vivo*. However, the effect was only slight. The same was true with the case of 3-aminophenoxazone-(2) derivatives examined by Hirata (1954). Koshimura (1952) also conducted

similar *in vitro* experiments to test 15 azo-compounds of aminophenol series and found that o, o'-dihydroxyazobenzene is most effective.

Sulphur compounds: Watanabe and others (1953, 1954) synthesized various sulphur compounds including 1, 4-dithiane to examine for antibacterial activity against tubercle bacilli in Kirchner liquid medium and found that the presence of sulphur in the structure of those compounds is concerned with the antituberculous activity. On the other hand, Takeda and his associates (1954) checked the antituberculous activity of thioamide derivatives. The minimum inhibitory concentrations were as follows; 10 μg per cc in the case of isonicotinic acid thioamide, 20 μg per cc in picolinic acid thioamide, 100 μg per cc in nicotinic acid thioamide, 50 μg per cc in p-aminothiobenzamide, and 100 μg per cc in furan- α -carboxylic acid thioamide. Taniyama and others (1954) studied on the *in vitro* antituberculous activity of thiazole and thiazolidone derivatives. 2-(P-aminobenzenesulfonyl)-hydrazino-4-thiazolidone, 2-(p-aminobenzenesulfonyl)-hydrazino-5-methyl-4-thiazolidone, and 2-(p-aminobenzenesulfonyl)-hydrazino-5-dimethyl-4-thiazolidone were found to be more effective than the similar thiazole derivatives. NH.NH_2 in the 2 position was essential for antituberculous activity and its replacement with NH resulted in loss of the activity. 2-Arylidenehydrazino-thiazole and its -4-methyl derivatives were mostly inactive, but 2-salicylidenehydrazino-thiazole and its -4-methyl derivatives showed a high growth-inhibitory activity. Ever since, they have been working on the relation between antituberculous activity and chemical structure of various thiazolidone derivatives (Tanihama, *et al.*, 1956). Kato and Yanagisawa (1954) conducted mice protection tests against tuberculous infection using thiourea and some thiazolidone derivatives which are highly active *in vitro* against tubercle bacilli. The result showed, however, that those compounds have not any antituberculous activity *in vivo*. Takada (1954) replaced pyridin nucleus of isoniazid with thiazol to make thiazolcarbonic acid hydrazide and its 6 derivatives. However, the antibacterial action against tubercle bacilli of these compounds were proved to be much weaker than isoniazid.

Hyrazine derivatives: Thirty years ago, Aoki (1928) found a very interesting fact that the development of experimental guinea pig tuberculosis was distinctly inhibited by the administration of phenylhyrazine. Later, Shirai (1930) tested various derivatives of phenylhyrazine for antituberculous activity. However, this series of compounds showed a strong hemotoxic action to cause anemia. For this reason, the research was abandoned thereafter. Recently, those former observations led Takeda and his associates again to an attempt to prepare more effective and less toxic hydrazine derivatives. Many compounds were prepared and some of them were applied to experimental guinea pig tuberculosis. However, if any, the effect was only slight, although hemotoxic action was decreased. p-Hyrazinophenylsulfonamide hydrochloride (Takeda *et al.*, 1950), 2-(p-hyrazinophenylsulfonamide) thiazole (Takeda *et al.*, 1952) and p-glucosylhyrazinophenylsulfonamide (Takeda *et al.*, 1950) were the representatives ever

tested. Soon after these works, news came from the United States reporting that isonicotinic acid hydrazide exerted a dramatic effect on human tuberculosis.

Phenazine derivatives: Kawakami and his co-workers (1955) examined 36 compounds of phenazine derivatives for *in vitro* bacteriostatic activity against tubercle bacilli. 1-Hydroxy phenazine di-N-oxide and iodinine were proved to be most effective in almost the same grade as PAS.

Naphthoquinone derivatives: Toda and Abe (1949) examined 14 naphthoquinone derivatives for *in vitro* antituberculous activity by their slide culture technique. They concluded that the compounds with low oxidation-reduction potential (E_0) are much more effective than those of high potential. Later, Yoda (1952) tested 17 derivatives of 1,4-naphthoquinone substituted in 2- and 3- position. The result indicated that 2-amino-1,4-naphthoquinone, 2-amino-3-chloro-1,4-naphthoquinone and 2-bromo-3-methyl-thio-1,4-naphthoquinone are effective. More recently, Hori (1953) published a summarized report of his own study of the antituberculous activity of naphthoquinone derivatives which had been conducted for many years. These substances were taken up by him on the idea that they are related with phthiocol in chemical structure. Forty-four agents including 18 new compounds were tested for antituberculous activity against H37Rv tubercle bacilli in Kirchner liquid medium. Almost all of them were insoluble in water, therefore they were added into the medium after being solved with propylene glycol. 1,4-Naphthoquinone, 2-acetylamino-1,4-naphthoquinone, 2-methylmercapto-1,4-naphthoquinone, 2-methyl-3-laurylmercapto-1,4-naphthoquinone and 2,6,7-trimethyl-1,4-naphthoquinone oxide inhibited the growth of H37Rv at less than 5 μ g per cc. However, these agents were found not so active *in vivo*.

Long-chained alkyl compounds: Nozu *et al.* (1953) examined 11 long-chained alkyl compounds for antituberculous activity *in vitro* with special reference to the relation between chemical structure and the activity. In the O-alkyl compounds, the activity was intensified to the certain maximum point with the increase of the chain length and then decreased contrarily, but such relation was not observed in the N-alkyl compounds. Similar studies were also conducted by Takada (1953) against H37Rv tubercle bacilli in Kirchner liquid medium. However, as pointed out by Hosonuma (1949), this series of compounds appear toxic to animals.

Quinoline derivatives: Six isomers of oxyquinoline were examined by Ueda and others (1953) for antituberculous activity *in vitro*. The activity was demonstrated only in the case that OH occupies the 8 position. The ester and the ammonium salt were devoid of the activity. Mono- and di-methyl derivatives were also ineffective.

p-Acetylaminobenzaldehyde thiosemicarbazone derivatives (Tibion series): Thirty compounds of tibion series were examined by Adachi and Kawakami (1953) for *in vitro* antituberculous activity against H37Rv tubercle bacilli. It

was found that the activity is not controlled by the sort of aldehyde group included, and no agent was more active than Tibion.

Allyl compounds: $\text{CH}_2\text{CH}:\text{CH}_2$ are attached to OH of phenol, thiophenol, cresol, thymol, guajacol and aminophenol to form ether. Endo (1954) examined antibacterial action against tubercle bacilli of these compounds and observed that allylether of m- or p-aminophenol and 2 allylaminophenol inhibited the *in vitro* growth of tubercle bacilli at 10 μg per cc.

Acryl acid derivatives: Endo studied various acryl acid derivatives but no effective agent was discovered.

Selene compounds: Maki (1954) tested 13 kinds of selene compounds for tuberculo-bacteriostatic activity *in vitro* against 7 strains of acid fast bacilli. The following three were most active against hyman type tubercle bacilli; 4-oxohomocampher-3,4-oxaselenazol-2-oxim, 5,5-dimethyl-7-nitroso- $\Delta^{3,7}$ -dihydro-benz-2,1,3-oxaselenazol and 4-oxo-homocampher-anhydro-selenoxyd. Furthermore, Nakamura (1954) treated experimental guinea pig tuberculosis with the first drug and Maki (1954) did with the second drug. However, both of them were too toxic to be administered in a sufficient amount and the efficacy was not satisfactory.

Furan and nitrofuran derivatives: Toda and others (1953) surveyed 9 nitrofuran derivatives for tuberculo-bacteriostatic activity *in vitro*. It was found that 2-(5-nitro)-furylacrolein semicarbazone and 2-(5-nitro)-furylacrolein thio-semicarbazone can inhibit the growth of tubercle bacilli at the 1:40,000 to 1:80,000 dilution. Nakamura (1954) in the same laboratory examined additional 12 nitro-furan derivatives to find that phenyl-5-nitrofurylacryloate is active at the 1:100,000 dilution. On the other hand, Uesaka and others (1953) observed that the growth of tubercle bacilli is inhibited by 5-nitrofural doxim in a dilution of 1:2,048,000, by 5-nitrofuryl acrolein oxim in a dilution of 1:512,000, and by 5-nitro-2-furfuryliden amino guanidine hydrochloride in 1:256,000. However, these agents were not significantly active against experimental tuberculosis (Uesaka *et al.*, 1953) (Niwata, 1954).

In Summary

As consolidated above, there has been presented many agents as the result of the screening test to search for newer active agents against tubercle bacilli. Chemotherapeutic agents and antibiotics act in most cases as bacteriostatic agents, blocking some essential metabolic pathway to put bacterial metabolism in disorder. In this point, they are different from antiseptics in general. Therefore, it is not unreasonable to think that many agents are to be discovered which act to inhibit the growth of tubercle bacilli each attacking different metabolic pathways of highly differentiated bacterial cell organization. On the other hand, bacterial resistance to chemotherapeutic agents and antibiotics most easily occurs in tubercle bacilli, particularly in the case of the long-term drug administration to this extremely chronic disease. If there are many agents ready to use at hand

for tuberculosis chemotherapy, there would be the brighter prospect of the future of the antituberculous campaign of human beings. Although the recent progress in tuberculosis chemotherapy has been quite remarkable, the ideal drugs have not yet been discovered which assure us of the complete bacteriological cure of human tuberculosis. Accordingly, our persistent endeavour appears to be requested as well as in the future.

In this review the present author presented a sketch of the recent progress of tuberculosis chemotherapy in Japan, citing various antibiotics and chemical compounds ever tried experimentally. Almost all of them, except kanamycin, had no practical value. However, the experiences that researchers of this country have had in such studies were never worthless. For instance, pyridomycin study indicated Umezawa and his coworkers that water-soluble antibiotics, however active they may be *in vitro*, would not be active *in vivo*. This recognition led them to search for water-soluble new antibiotics, particularly for those basic and of less-toxic nature. Kanamycin has finally been discovered by them under such idea.

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