

ABSTRACTS

1. FUNDAMENTAL STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS

PART 68. EXPERIMENTS ON MICE

NO. 1. CURATIVE EFFECT OF SINGLE ADMINISTRATION OF ANTITUBERCULOUS AGENTS

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Administering, singly, SM, PAS, INAH and o-aminophenol (OM) to tuberculous mice the author obtained the following results :

- 1) As to efficacy, the drugs seem to have the relation,
$$\text{INAH} \geq \text{SM} > \text{OM} \geq \text{PAS}.$$
- 2) The tubercle bacilli in the animals' viscera gradually decreased in number as administration of SM or INAH was continued, but complete eradication was never observed.
- 3) Drug-resistant bacilli appeared only in small numbers and had only a low degree of resistance.
- 4) The results of administration of OM to tuberculous animals did not significantly differ between mice and guinea pigs.

2. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 17. ON THE ANAMNESTIC SERUM REACTIONS OF RABBITS IMMUNIZED WITH OT-SENSITIZED RED BLOOD CELLS

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As a part of the study on immunization against tuberculosis, the anamnestic reactions of rabbits immunized with OT-sensitized red blood cells toward specific and non-specific stimuli were observed, including agglutination and hemolysis of OT-sensitized red cells, agglutination of OT-sensitized red cells pretreated with tannic acid, *Inoue's* bacilli agglutination, precipitation and complement fixation reactions. The animals were given a second injection of OT-sensitized red cells, an injection of typhoid fever vaccine or X-ray irradiation when the antibody titer resulting from the initial injection had become low. Observation of the anamnestic serum

reactions and phagocytosis induced thereby gave the following results :

1. The anamnestic serum reactions appeared no quicker than after the initial injection and the antibody titer was lower.
2. Of the three treatments, re-injection of the same immunizing agent gave the strongest anamnestic reactions of all kinds, and moreover showed some reduction of the negative period of phagocytosis.
3. When typhoid fever vaccine was injected, no anamnestic precipitation or complement fixation reaction was observed, and the other reactions were very weak, except the phagocytosis which developed in the same way as when OT-sensitized red cells were injected again.
4. In the group receiving X-ray irradiation :
 - i) Definite anamnestic reaction was recognized about agglutination and hemolysis of OT-sensitized red cells and agglutination of similar cells pretreated with tannic acid. Each reaction was strongest when the dosage was 50 r, less strong at 200 r and very weak at 600 r.
 - ii) Anamnestic *Inoue's* bacilli agglutination and complement fixation reactions were noticed only at 50 r.
 - iii) No anamnestic precipitation reaction was recognized at all.
 - iv) About phagocytosis, 50 r and 200 r irradiation produced effects comparable to those produced by re-injection of OT-sensitized red cells, but at 600 r, only the direct effect of the irradiation was observable.

3. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTE

PART 5. STUDIES ON ERYTHROCYTE SENSITIZING SUBSTANCES IN OLD TUBERCULIN (2)

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In the previous experiments, all the 4 fractions from OT, i. e. two protein fractions (PF_I and PF_{II}) and two polysaccharide fractions (CF_I and CF_{II}), were proved to have erythrocyte-sensitizing potency.

In the present study, the serum of a rabbit receiving intravenous injection of its red cells sensitized with OT or each of the four fractions was tested for its power of hemagglutination and hemolysis.

The results obtained were as follows :

- 1) All the fractions have the power, stronger or weaker according to the fraction, of producing antibodies, which, however, was weaker than the power of OT.

2) From the data of absorption tests of the antiserum produced with OT-sensitized red cells it was found that the erythrocyte-sensitizing power of OT was not due to a single substance.

4. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 8. MECHANISM OF ACQUISITION AND LOSS OF DRUG-RESISTANCE BY BACTERIA

No. 2. EXPERIMENTS WITH *MYCO. 607* AND *MYCO. PHLEI*

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In the preceding study, a cross relation was proved between the cell membrane permeability of *Myco. avium* (Takeo strain) and its SM-sensitivity.

In the present experiment, attempts were made to investigate whether the SM-sensitivity of *Myco. 607*, *Myco. phlei* and their SM-resistant strains is affected by the same conditions.

The results obtained were as follows :

1) The SM-sensitivity of *Myco. 607* decreased when cultivated in modified Sauton's medium containing 15% glycerol, and that of *Myco. 607* SM-resistant strain increased when heating and freezing operation on the bacilli was performed in bacillary body extract of *Myco. 607*. On the other hand, SM-sensitivity of *Myco. phlei* and its SM-resistant strain was not affected by the respective processes.

2) The bacillary body extract of *Myco. avium* and that of *Myco. 607* were observed to increase the drug-sensitivity of SM-resistant strain of the respective organisms but not of SM-resistant *Myco. phlei*. Moreover the bacillary body extract of *Myco. phlei* was not found to increase the drug-sensitivity of any of the three SM-resistant strains.

5. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 8. MECHANISM OF ACQUISITION AND LOSS OF DRUG-RESISTANCE BY BACTERIA

No. 3. ADDITIONAL EXPERIMENTS WITH *MYCO. 607* AND *MYCO. PHLEI*

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In the preceding study, SM-sensitivity was observed to change in *Myco. 607* and its SM-resistant strain but not in *Myco. phlei* or its SM-resistant strain under the experimental condition which presumed to change the cell membrane permeability of *Myco. avium* and its SM-resistant strain.

In the present study, attempts have been made to investigate whether there exist experimental conditions which affect the SM-sensitivity of *Myco. phlei* and its SM-resistant strain, or conditions which affect the SM-sensitivity of *Myco. 607* and its SM-resistant strain more strongly than those conditions adopted in the previous experiment.

- 1) SM-resistance of *Myco. 607* "SM-resistant" strain and *Myco. phlei* "SM-resistant" strain decreased by successive culture in modified Sauton's medium containing 0.5 % glycerol.
- 2) The power of bacillary body extract of *Myco. 607* of reducing the SM-resistance of the resistant strain of the same bacillus was observed to have close relation with the kind of pH and temperature of the extracting fluid, the length of time spent in the extracting and the period in which the extract is kept in the ice-box, but to be independent of the length of the culture period ranging from 2 to 21 days.
- 3) The SM-resistance of the resistant strain of *Myco. phlei* was not observed to be decreased by the bacillary body extract of *Myco. phlei* prepared under any of the conditions employed.

6. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 10. STUDIES ON THE DOUBLY DRUG-RESISTANT TUBERCLE BACILLI NO. 1. DEVELOPMENT OF RESISTANCE AGAINST OTHER ANTITUBERCULOUS AGENTS IN SM-RESISTANT AND INAH-RESISTANT STRAINS OF HUMAN TUBERCLE BACILLI "H₂"

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Human tubercle bacilli "H₂" strain and its SM-resistant and INAH-resistant strains were successively cultured on Ogawa's 1 % KH₂ PO₄ egg yolk medium containing various concentrations of SM, PAS, INAH, IHMS, Tbl, VM or o-aminophenol (OM), and the pattern of

resistance development of the bacilli against the each agent was compared, and sensitivity to the other agents of the doubly drug-resistant strains thus obtained was measured.

The results obtained were summarized as follows.

- 1) The SM-resistant and the INAH-resistant strains developed high resistance to the other antituberculous agents contained in the medium.
- 2) All the three strains were observed to develop only low resistance (100~500 γ /ml) to chemical compounds such as PAS, Tbl, INAH and IHMS, but high resistance (over 20,000 γ /ml) to antibiotics such as SM and VM.
- 3) None of the three strains acquired resistance to OM.
- 4) The patterns of development of resistance to INAH were quite similar to those against IHMS in the SM-resistant strain and the susceptible "H₂" strain.
- 5) The SM-resistant and INAH-resistant strains do not show any lowering of the resistance when subjected to successive culture on the medium containing a different antituberculous agent.
- 6) The INAH-resistant strain was observed to have low sensitivity to SM.
- 7) The INAH-(or IHMS-) resistant strain was proved to have cross resistance to PZA.
- 8) The bacilli resistant to VM plus INAH were proved to have cross resistance to SM of the order of 100 γ /ml.

7. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 10. STUDIES ON THE DOUBLY DRUG-RESISTANT TUBERCLE BACILLI No. 2. VIRULENCE OF DOUBLY DRUG-RESISTANT STRAINS UPON MICE

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In the previous experiments, singly and doubly drug-resistant tubercle bacilli were isolated after successive culture of the SM-resistant and INAH-resistant strains and the susceptible parent strain H₂ on 1% Ogawa's solid medium containing antituberculous agents such as SM, PAS, Tbl, INAH, IHMS, VM and o-aminophenol (OM).

In the present study, the virulence of the original three strains and the isolated strains was tested on mice by means of intraperitoneal infection.

The results obtained were summarized as follows :

- 1) The virulence of the SM-resistant strain was found to be almost equal to, and that of the INAH-resistant strain somewhat lower than, that of the parent H₂ strain.

2) The virulence of the INAH-SM-resistant strain was almost the same whether it was derived from the SM-resistant strain or the INAH-resistant one, and little lower than that of the SM-resistant strain.

3) The original three strains were found to develop VM-resistance with a little increase of the virulence.

4) The virulence of INAH-resistant strain was found to be equal to that of IHMS-resistant one, and that of SM-INAH-resistant strain to that of SM-IHMS-resistant one.

5) The three original strains developed PAS or Tbl resistance without any change of the virulence.

8. A CERTAIN NON-ACID-FAST BACILLUS PRODUCING A TUBERCULIN-LIKE SUBSTANCE

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The author isolated from a sample of sputum collected on the street surface of Kanazawa City in October 1956 a certain non-acid-fast microbe which on cultivation produced a substance resembling old tuberculin, and studying its properties obtained the following results :

1. It is a polymorphic rod, non-motile and non-sporogenic, having no capsule or flagella. It is stained with common dyes but is not acid-fast, the Kf being below 0.3. It is gram-positive and under special circumstances shows dipolar staining.

2. It grows on ordinary culture media. On solid media it generally assumes the S type and less frequently the R type. It grows vigorously in media enriched with glycerine such as Sauton's and produces a brick-red pigment. It is scotochromogenous and produce indole, gives negative Dubos' neutral red reaction but strongly positive catalase reaction, and decomposes sorbit. The growth is most vigorous at 37° C and stops at 19° C. The optimum pH range is 7.2~8.6 and the growth range, 5.4~9.2.

3. It is susceptible to SM and penicillin, but resistant to PAS, INAH, Tbl and OM.

4. It is non-pathogenic to the mouse and the rabbit.

5. Antigenicity.

1) Production of tuberculin-like substance.

A substance (OT "16") which is obtained from the culture filtrate (Sauton's medium) of No. 16 by means of the technique for obtaining old tuberculin, induces positive skin reaction in rabbits infected with human type tubercle bacillus "H₃₇ Rv" or with No. 16.

2) The erythrocyte-sensitizing factors in OT "16" and OT "H₃₇ Rv".

The results of absorption tests carried out on sera of animals immunized with OT "16"-sensitized cells and animals immunized with OT "H₃₇Rv"-sensitized red cells indicate that the two "tuberculin" have antigens common to each other as well as antigens peculiar to each.

9. STUDIES ON ACID-FAST BACILLI IN SPUTA COLLECTED FROM STREET SURFACE

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Twenty-one acid-fast bacilli isolated from 108 sputa on the street surface of Kanazawa city in October 1956 were tested for their behavior under cultivation, dyeing property, catalysing power, cord-formation and sensitivity to drugs. And then the filtrates of culture media were examined for their power of causing skin reaction and sensitizing red cells.

The results obtained were as follows :

- 1) Eight out of the 21 strains were identified as human type tubercle bacilli. The remaining 13 were found nonpathogenic, but some of them showed in the tests carried out in this experiments, properties closely resembling those of human type tubercle bacilli.
- 2) It is worthy of notice that the pathogenic and the nonpathogenic bacilli could be positively distinguished by the degree of their sensitivity to o-aminophenol.
- 3) From the standpoint of epidemiology it was a significant fact that 4 of the 8 pathogenic bacilli were resistant to both SM and PAS.

10. HISTOLOGICAL STUDIES ON TUBERCULIN ALLERGY

PART 3. INFLUENCE OF DRUGS ON TUBERCULIN REACTIONS
NO. 4. INFLUENCE OF CORTISONE

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In order to study the influence of cortisone on tuberculin reaction, three groups of rabbits were sensitized with dead human type tubercle bacillus strain "H₃₇Rv" suspended

in liquid paraffin. Intramuscular injection of 25 mg of cortisone was given daily for 21 days to each animal, starting on the same day as the sensitization for group 1 and three weeks thereafter for group 2. No injection was given to group 3, which was used as control.

Observation of tuberculin reaction was made on all the groups at the end of each week following the sensitization, both with the naked eye and microscopically. Also the variation of the polysaccharide content and the vascular permeability of the localities were observed as well as the animals' body weight and the power of their sera to agglutinate OT-sensitized red blood cells. The tuberculins used were of three kinds, OT with and without addition of hyaluronidase and o-aminophenol azo-tuberculin "Human" (OA-Azo-T"Human"). The results obtained were as follows :

- 1) Administration of cortisone is generally accompanied with impaired appetite and diarrhea. Loss of body weight was observed in both groups, being more conspicuous in group 1.
- 2) The production of antibodies for agglutination of OT-sensitized red cells was considerably delayed in group 1, but not at all in group 2, compared with the control group.
- 3) The appearance of tuberculin reaction, as observed with the naked eye, was delayed by cortisone in group 1. Group 2, which showed regular positive reaction before the injection was started, showed weaker reaction afterward, the degree of weakening being most pronounced with OA-Azo-T"Human" and then OT with hyaluronidase and least pronounced with simple OT.
- 4) The exudation of polymorphonuclear leucocytes in the earlier tissue specimens as well as that of mononuclear leucocytes in later specimens was diminished by cortisone, the latter especially so with OA-Azo-T"Human".
- 5) The amount of polysaccharides present in the localities of the test was estimated by Lillie's method. Group 1 showed some decrease of the amount but group 2 did not show significant difference from the control.
- 6) Cortisone was seen to restrain the increase of the vascular permeability of the localities where the tuberculins were injected. In the control group the permeability was found to be higher in the locality receiving OT with hyaluronidase than that receiving simple OT.

11. STUDIES ON THE INFLUENCE OF PULMONARY CIRCULATORY DISTURBANCE UPON PULMONARY TUBERCULOUS LESION

PART 1. FORMATION OF EXPERIMENTAL TUBERCULOUS PULMONARY CAVITY

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The author was planning to study the influence of pulmonary circulatory disturbance

upon tuberculous pulmonary cavity, and for the first step he attempted to produce tuberculous cavity in dog's lung by means of inoculation of virulent bovine tubercle bacilli. The results obtained were as follows :

1) Although dog leant to be weak for getting tuberculin allergy, it was necessary for the efficient formation of the tuberculous pulmonary cavity that animal had been sensitized with BCG as a preliminary procedure. Actually, cavity formation of the lung resulted in higher rate in sensitized dog than in nonsensitized dog.

2) Out of various ways of invasion of bacilli, intrabronchial infection with adequate amount of bacillary suspension brought the best results in cavity formation. Describing technique of intrabronchial inoculation, ureteral catheter which contained thinner metal tube in it, was inserted into the bronchus under bronchoscopy and tubercle bacilli suspended with sodium alginate or jodic oil were poured in through the metal tube. When 5mg per 0.5ml of tubercle bacilli was inoculated by this method, tuberculous pulmonary cavity was made in 81 % of all the sensitized animals. The reason why cavity formation was accomplished in high percent by this method, was explained in the way that tubercle bacilli was localizingly inoculated without fail in the expected region of the bronchus and futile spreading of bacilli was avoided by high viscosity and specific gravity of jodic oil used for suspension.

3) Experimentally produced cavity of dog's lung was checked from 2 weeks to 12 months after inoculation. Most histological response in cavity wall was proliferation of epitheloid cell, and caseous necrosis as seen in human tuberculous lesion did scarcely appear in the tissue. This was due to low potency of tuberculin allergy of dog.

12. PATHOLOGICAL STUDIES ON THE TUBERCULOUS PULMONARY LESIONS AND THEIR DRAINAGE BRONCHI OF THE RESECTED SPECIMENS

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There have been lots of reports about pathological and bacteriological examination of resected specimens, however, few were ones that have checked minutely and systematically on the drainage bronchi of lesions such as tuberculous cavities, inspissated cavities, tuberculomas and encapsulated caseous lesions. The author put special attention on the patho-histological observation of the drainage bronchi of lesions of 100 resected specimens and obtained the following results.

1) Cavities were perceived in 54 cases out of 100, and increase of changes of the drainage bronchi was the greatest, compared with those of bronchi of other lesions. The closer the

segments of bronchi situated to cavities, and the filthier the cavities were, the greater the changes of bronchi were.

2) Open healing cavities were perceived in 4 cases, and the cavity wall was covered with non-specific granulation tissue which contained collagenous fibres and carried slight reepithelialization. No tubercle bacillus was detected in the tissue. Changes of the drainage bronchi were slight; there was no ulcer on the mucous membrane, reepithelialization was seen near bronchocavitary junction, and chronic inflammatory reaction was slightly present on the submucous tissue. The drainage bronchi were patent joining to the lobar bronchi.

3) Inspissated cavities were perceived in 28 cases out of 100, and changes of the drainage bronchi were great next to those of bronchi of cavities; in regard to ulcerous changes of the mucous membrane and some findings of the submucous tissue, there was no significant difference between bronchi of inspissated cavities and those of ordinary cavities.

4) Tuberculomas were perceived in 6 cases out of 100, and changes of their associated bronchi were very slight, comparing with those of bronchi of cavities or inspissated cavities. However, there were many cases in which considerable reaction of inflammation was seen on the submucous tissue of bronchi. Ulcerous change was also recognized on mucous membrane, although in only one case.

5) Encapsulated caseous lesions were perceived in 12 cases out of 100, and changes of their associated bronchi were slighter than those of bronchi of cavities and inspissated cavities and much more remarkable than those of associated bronchi of tuberculomas. Ulcer was present on mucous membrane although in but a few cases and inflammatory tissue reaction was seen on submucous layer along the entire length of the bronchus.

6) From viewpoint of change of the bronchus, cavity and inspissated cavity should be regarded as the same.

7) In tuberculoma and inspissated caseous lesion, change of the associated bronchus was slight in general, however, it should be noticed that there were some cases affected by malcondition such as ulcer of mucous membrane, submucous inflammation and stricture, of the bronchus.

8) The microscopical evidence showing that INH was effective on tuberculous lesion of mucous membrane of the drainage bronchus could not be ascertained by the findings of common cases, however, the findings of drainage bronchus of open healing cavity made assume that the drug was considerably effective on the disease of the mucous membrane.

13. HISTOCHEMICAL STUDIES ON TUBERCULOUS LESIONS OF RESECTED LUNGS

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Authors have performed histochemical studies on 84 resected pulmonary lesions obtained from 71 tuberculous patients by means of various staining techniques for enzymes' activities, fats and polysaccharides in the tissue. The results obtained were as follows :

1) The activity of various enzymes such as alkaline phosphatase, acid phosphatase, 5-nucleotidase, alkaline ribonucleotidase, acid ribonucleotidase, alkaline desoxyribonucleotidase, acid desoxyribonucleotidase, lipase and catalase was vigorously shown in the tissue of tuberculous cavities and inspissated cavities, while it was slackly showed in the tissue of encapsulated caseous lesions.

2) Polymorph nuclear leucocytes which were seen at the site of softening and liquefaction of caseous lesion, were usually endowed with vigorous activity of various enzymes, consequently they might play a important role in the process of softening and liquefaction of tuberculous lesion.

3) Fat and lipid were scarcely observed in the tissue of cavitory lesions and inspissated cavities, while they were settled in large amount around encapsulated caseous lesions, in particular, settled with significant density if lesions were smaller than the size of a lobulus and if the lesions showed pathologically stable state leaning to healing.

4) Polysaccharides were scarcely present in the tissue of tuberculous cavities and inspissated cavities, however, they were much observed in the tissue of encapsulated caseous lesions. Polysaccharides were densely deposited also into the connective tissue fibres of capsules around caseous lesions ; this process would help to form encapsulation of the lesions, accordingly it had a close relation to the stabilization and improvement of the tuberculous lesions.

14. STUDIES ON OPEN NEGATIVE SYNDROME

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Authors had performed clinical examinations of 307 patients of pulmonary tuberculosis who underwent resectional therapy of the lung, and successively made precise pathological and bacteriological researches on the resected specimens after surgery. Based on the findings

above studied, they could inquire into the particular cases with open negative syndrome which was characterized by the simultaneous existence of cavitation on X-ray and negative sputum of tubercle bacilli. The results summarized were as follows :

1) There were 34 cases (corresponding to 11.6 percent of all) with open negative syndrome, in which rentogenography showed clearly cavitation and sputum was revealed negative on serial cultivations for more than 6 months before surgery.

2) Patients with open negative syndrome did scarcely show acute clinical symptoms.

3) Patients with open negative syndrome were featured by the rentogenographic evidences mostly showing sclerotic wall cavities and tuberculomas contained softening within them.

4) At the time of starting chemotherapy, 7 cases out of 34 with open negative syndrome had had positive sputum of tubercle bacilli, while in 6 sputum turned into negative after chemotherapy for 1~3 months and in 1 resistant strain of streptomycin was detected in sputum which took negative conversion employing injection of isonicotinic acid hydrazide and o-aminophenol into the cavity.

5) Bacteriological examination of resected specimens of the syndrome revealed positive in tubercle bacilli in 13 cases, corresponding to 38.2 percent of the cases with open negative syndrome. This fact suggested that the patients with the syndrome were exposed themselves to danger of relapse of the disease.

6) Pathological examination of 34 specimens obtained from the patients with the syndrome, brought the following arrangement ; 5 of open healing cavities, 12 of caseous cavities, 6 of inspissated cavities, 9 of encapsulated caseous lesions, and 2 of emphysematous bullae.

7) Open healing cavities showed different histological evidences from most lesions of tuberculosis ; granulation tissue which covered inner wall of cavities, was completely free from caseous material, moreover it was rich in collagenous fibres and metaplasia of squamous epithelium partially developed in the tissue. Amount of collagenous fibres of granulation tissue was quite different, showing various conditions from starting formation of fibre to scar tissue which was regarded as complete replacement of the connective tissue. No tubercle bacillus was detected in the tissue of open healing cavity.

8) Patients with open negative syndrome should be operated upon with resection, judging from bacteriological and pathological findings of the lesions.