

ABSTRACTS

1. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 20. INFLUENCE OF TUBERCULIN FRACTIONS UPON THE TISSUE RESPIRATION OF TUBERCULOUS MICE

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The following 3 groups of mice were subjected to experiment to investigate the influence of fractions of OT upon the tissue respiration of tuberculous organs.

Group A. Mice infected with human tubercle bacilli "H₃₇Rv"

Group B. Mice treated with heat-killed human tubercle bacilli suspended in liquid paraffin oil

Group C. Untreated mice as control

The results obtained are summarized as follows :

1) The tissue respiration of Groups A, C and B was observed to increase in that order by addition of OT in the medium.

2) The protein fraction from OT accelerated the tissue respiration of Groups A and C, and suppressed that of Group B.

3) The polysaccharide fraction from OT accelerated the tissue respiration of Group A, but did not affect that of Group B or C.

4) O-aminophenol azo-tuberculin prepared from OT produced little change in the tissue respiration of any of the groups.

2. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 21. SIGNIFICANCE OF ADJUVANTS IN IMMUNIZATION

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For the purpose of clarifying the mechanism of the adjuvant effect, the following 3 groups of guinea pigs were employed in the experiment.

Group A. Guinea pigs treated with heat-killed human tubercle bacilli suspended in liquid paraffin oil

Group B. Guinea pigs treated with heat-killed human tubercle bacilli suspended in saline

Group C. Guinea pigs treated with liquid paraffin oil

The results obtained are summarized as follows :

1) Development of tuberculin skin reaction and production of Middlebrook-Dubos antibody were observed only in Group A two or three weeks after the treatment.

2) The change of the visceral organs was most serious macroscopically and pathologically in Group A.

3) The bacilli injected were observed to stay at the injected site and the viscera far longer in Group A than in Group B.

3. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 22. STUDIES OF THE INFLUENCE OF DESENSITIZATION ON TISSUE RESPIRATION

No. 2. EXPERIMENT IN DESENSITIZATION WITH o-AMINOPHENOL AZO-TUBERCULIN

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The following 5 groups of mice were subjected to experiment to investigate the influence of desensitization with o-aminophenol azo-tuberculin (OA-Azo-T) upon tissue respiration.

Group A. Mice sensitized with heat-killed human tubercle bacilli suspended in liquid paraffin oil

Group B. Similarly sensitized mice receiving afterward daily injection of OA-Azo-T

Group C. Similarly sensitized mice receiving afterward daily injection of OT

Group D. Unsensitized mice receiving daily injection of OA-Azo-T

Group E. Untreated mice as control

The results obtained were as follows :

1) No difference of tissue respiration was observed between Groups D and E, but when OT or OA-Azo-T was added to the medium, increase of the respiration was observed in both groups, much more marked in Group D than in Group E.

2) A same degree of increase of respiration was observed in Groups A, B and C as compared with Group E.

When OT was added to the medium, increase of the respiration was observed in Groups

A, B and C, more marked in Groups A and B than in Group C.

When OA-Azo-T was added to the medium, the respiration was observed to increase in Groups A and D, to decrease in Group C and to remain about the same in Group B.

4. IMMUNOLOGICAL STUDIES OF OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 18. ON THE PROPERTY OF AFFORDING PROTECTION
AGAINST CHALLENGE INFECTION

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OT-sensitized erythrocytes were tested for their ability of protecting guinea pigs against challenge infection.

The results obtained were as follows :

1) The pretreatment with OT-sensitized erythrocytes produced Middlebrook-Dubos antibody but induced no tuberculin skin allergy in the animals.

2) Little protective effect of the pretreatment was observed in the animals either by macroscopical examination of the viscera or by quantitative culture of the bacilli present in the visceral organs.

5. STUDIES ON RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 13. MECHANISM OF ACQUISITION OF INAH-RESISTANCE
BY *Mycobacterium avium*

No. 2. DECOMPOSITION OF INAH BY VARIOUS STRAINS OF
Mycobacterium Avium AND INFLUENCE OF
DRUGS UPON THE DECOMPOSITION

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For the purpose of clarifying the mechanism of acquisition of INAH-resistance by a

mycobacterium, a series of experiments was carried out concerning the decomposition of INAH by *M. avium* "Takeo" strain, and the results were already reported in a previous paper.

In the present study, "Jucho", "Hosoya", "A 62" and "4127" strains of *M. avium* were employed in place of "Takeo" strain.

Furthermore, the influence of various drugs upon the decomposition of INAH by *M. avium* "Takeo" strain was observed.

1) All the strains used were almost equal to *M. avium* "Takeo" strain in the activity of decomposing INAH.

2) VB₂ and VK suppressed the decomposition of INAH by *M. avium* "Takeo" strain.

6. STUDIES ON RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 13. MECHANISM OF ACQUISITION OF INAH-RESISTANCE

BY *Mycobacterium Avium*

No. 3. DECOMPOSITION OF INAH BY *Mycobacterium*

Tuberculosis Var. Hominis

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In a previous paper, the author reported the results of experiments concerning the decomposition of INAH by *M. avium*.

In the present study, similar experiments were carried out with *M. tuberculosis var. hominis* in place of *M. avium*.

The results obtained are summarized as follows :

1) *M. Tuberculosis var. hominis* was observed to decompose INAH, the strain resistant to the drug much more rapidly than the sensitive strain.

2) The living bacilli, heat-killed bacilli and the lipid in them were equally potent in decomposing INAH, but defatted bacilli were observed to be almost totally inactive.

3) The INAH-decomposing activity of *M. tuberculosis var. hominis* was slightly lower than that of *M. avium*.

It must be admitted that the difference of the activity may have been due to the difference between the growing rates of the two mycobacteria.

7. ISOLATION OF CANDIDA GROUPS FROM PATIENTS
WITH PULMONARY TUBERCULOSIS

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Candida were isolated from a large percentage of the sputa of patients with pulmonary tuberculosis over 40 years of age and of patients with positive sputum of SM-resistant tubercle bacilli. The majority of the isolated candida was *C. alb.*, and the rest consisted of *C. trop.*, *C. pseudotrop.*, *C. Krusei* and *C. parakrusei*.

8. CYTOLOGICAL STUDIES ON THE EXPERIMENTAL
TUBERCULOUS INFLAMMATION

No. 3 CELLULAR REACTIONS BY THE INJECTION OF MINIMAL DOSES
OF SEMI-DRIED HEAT-KILLED MYCOBACTERIA OF HUMAN
STRAIN, H₃₇Rv AND BOVINE STRAIN, B C G

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Periodical observation was made of the cellular reaction caused by injection of minimal doses of semi-dried heat-killed (at 100°C for 30 minutes) mycobacteria, H₃₇Rv and B C G. in the subcutaneous tissue of mice.

The following results were obtained :

1. Cellular reactions of almost the same degree were observed to be caused by injection of dead bacilli of the human and the bovine strain throughout the whole period of inflammation.

2. The proliferation of small mononuclear cells induced by the dead bacilli proceeded to a limited extent, while the intensity of proliferation of the cells induced by the virulent living tubercle bacilli was more pronounced and more durable.

Hypertrophia of the cells and their cellular accumulation were, however, more frequently

observed when dead bacilli were used than when virulent living mycobacteria were employed.

3. Appearance of epithelioid and giant cells was brought about more frequently by the dead bacilli than by the living.

4. It was seen that tubercle bacilli were gradually digested in the cytoplasm of the cells of epithelioid, giant and intermediate types.

9. CYTOLOGICAL STUDIES ON THE EXPERIMENTAL TUBERCULOUS INFLAMMATION

No. 4 CYTOLOGICAL STUDIES ON THE SMALL MONONUCLEAR, EPITHELIOID AND GIANT CELLS

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Experiments were carried out with semi-dried living B C G, the same procedures being employed as in No. 1 of this series of studies. To study the cytological character of the cells appearing in the inflammatory area by the injection, the following staining methods were employed : May-Giemsa, vital, supravital, peroxydase, neutral osmic acid, sudan and silver nitrate (0.1 %).

The results obtained were as follows :

1. The small mononuclear cells, mesenchymal in origin, are young histiocytes, and some of them develop into epithelioid cells or giant cells. The giant cells probably result from amitosis of the nucleus unaccompanied by division of the protoplasm.

These cells play a principal role in the tuberculous inflammatory process.

2. The epithelioid cells and giant cells contain abundant mitochondria.
3. They show powerful phagocytic activity toward tubercle bacilli, both living and dead.
4. It was observed that living mycobacteria caused intensive proliferation of small mononuclear cells, while killed bacteria induced appearance of epithelioid, and giant cells in abundance and weak proliferation of the small mononuclear cells.

10. STUDY OF EXPERIMENTAL PULMONARY TUBERCULOSIS IN RABBITS, ABOUT RELATIONS BETWEEN THE PRIMARY LESION AND SECONDARY LESION IN THE LUNG AND THE RELATED BLOOD VESSEL CHANGES

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It is a well-known fact that histopathological changes are modified by immunity and allergy, but in the cases of human chronic pulmonary tuberculosis the relation is too complicated to identify experimentally.

The author tried to differentiate between primary and secondary tuberculosis in the following experimental groups in which the inoculations were made in different localities by means of the adjuvant method.

The groups were as follows;

1. The first inoculation was performed by direct injection of tubercle bacilli through an intercostal space on one side and the second inoculation on the other side.
2. The first inoculation was made intratracheally and the second by direct intrapulmonary injection.
3. The first was made by intracutaneous injection in the thigh and the second by direct intrapulmonary injection.
4. Same as group 3, but with the order reversed.

The experimental animal was the rabbit and the tubercle bacilli were the bovine type of Miwa's strain.

The adjuvant was a mixture of 3 parts fluid paraffin and 1 part lanolin dehydrate and 10 % gelatin.

The experimental results were as follows.

1) The lesions produced by direct pulmonary injection of the tubercle bacilli with the adjuvant show non-specific necrosis and bleeding with slight epithelioid cell reaction in the peripheral area. When the same material is injected into the other lung, the primary lesions reveal regression of the primary central necrosis with relatively strong epithelioid cell reaction, but when large amounts of tubercle bacilli are injected strong caseation is sometimes produced in the primary foci. The same phenomena are observed when the secondary injection is made subcutaneously in the thigh.

2) When the tubercle bacilli are injected into the trachea, small, scattered tubercle foci with slightly caseous tendency are produced. These primary foci are hardly influenced at all by the secondary tuberculosis in other localities, but tend rather to healing or resorption. The secondary pulmonary tuberculous foci produced by direct intrapulmonary inoculation subsequent to production of extrapulmonary primary tuberculosis, show cavity formation by non-specific necrosis.

In the experiment with group 1, the secondary intrapulmonary foci reveal the same tendency, but the epithelioid cell reaction is stronger than in group 2.

3) The vascular changes of the lung in those experimental groups, are considered to be due to the secondary effects of the tuberculous infection and the damages resulting from the adjuvant.

4) Administration of INAH makes the acute exudation of the pulmonary blood vessels disappear, but has hardly any effect on relatively chronic vascular changes.

5) The pulmonary cavity produced by Yamamura's method, show relatively strong inhibition when INAH is administered. This fact certainly indicates that the drug affects the activity of the bacilli in the secondary foci. But the reduction of the allergic influence of the primary foci on the secondary due to the alleviation or suppression of the infection at the primary foci is considered to be an important factor too.

Publications not appearing in the Ann. Rep. Tbc.

Kanazawa (1960)

1) **Ito, R. and Hosokawa, K.** : Inhibition of Streptolysin S by Tubercle Bacilli

Data were presented to show that when suspended in saline, the organisms of certain strains of *Mycobacteria*, including human and bovine tubercle bacilli, exert inhibition of Streptolysin S, and that this inhibition of the toxin is attributable to the action of a soluble substance(s) which is released upon disrupting the cultural mass of *Mycobacteria*.

The organisms of *Mycobacteria*, such as *M. avium*, *M. phlei*, *M. tuberculosis* 607 and a chromogenic acid-fast bacillus Ishii, did not share the effect against Streptolysin S.

The anti-hemolytic activity of tubercle bacilli greatly varies with strains, the organisms of such human strains H₂, Frankfurt and H₃₇Ra, exhibited a well marked inhibition of the toxin, while those of H₃₇Rv, Kawakami and Aoyama B, and the two bovine strains No. 10 and BCG, all were found to be less active in inhibitory effect.

The age of culture and the kinds of culture media on which the bacilli were grown did not affect the anti-hemolytic activity.

Streptomycin-resistant bacilli did not differ in the anti-hemolytic activity from the original streptomycin-sensitive strains.

The soluble substance responsible for the anti-hemolytic activity of tubercle bacilli was non-dialysable, destroyed by heating at 100°C for 30 minutes, and readily inactivated by pepsin, though quite resistant to the action of trypsin, papain, lipase and amylase.

Among a variety of bacteria other than *Mycobacteria*, *B. subtilis* or *mesentericus* was found to be the organism inhibiting Streptolysin S.

Japan. J. Tuberc., 7, 1-9, 1959.

2) **Ito, R., Matsuda, M. and Aoki, K.** : Studies on Effect of Tannic Acid on Streptolysin-S Susceptibility of Erythrocytes.

1. It was demonstrated that guinea pig erythrocytes, when treated with suitable concentration of tannic acid, rapidly lost their susceptibility to the lytic action of Streptolysin-S. Further experiments showed that erythrocytes of different species were not equally affected by tannic acid: thus, red cells of ox, sheep, and goat were rendered thereby highly resistant to the toxin, and those of guinea pig, mouse, dog, pig and cat rendered more or less insusceptible, whereas cells of the rabbit remained unaffected.
2. The tannic acid effect was reversible in the sense that the lost susceptibility of tannic acid-treated erythrocytes was rapidly restored on exposure to certain proteins. Of proteins studied, gelatin was most active, followed by albumin and γ -globulin.

Among other substances tested, chondroitin sulfate and mucin were also found to be considerably active.

Jap. J. Pharmacol., 9, 169-174, 1960.

3) **Koshimura, S., Shimizu, R., Bando, Y., Hirata, R. and Shoin, S. :**

Experimental Anticancer Studies. Part 12. On the Destructive Effect of Living Hemolytic Streptococci on Solid Tumor of Ehrlich Carcinoma in Mice

The communication deals with the results obtained from the experiment, in which mice with solid tumor of Ehrlich carcinoma were intratumorally injected with living hemolytic streptococci.

The principal results were as follows.

1. The most striking change occurred in all animals which received living hemolytic streptococci was the rapid softening and destruction of the tumor mass, followed by formation of ulcer.
2. On the contrary, any actual destruction of tumor mass has not occurred in the experimental animals which received heat-killed streptococci.
3. Additionally, appreciable amounts of Streptolysin-S were detected in solid tumors receiving the living cocci.

Japan. J. Microb., 4, 19, 1960.