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No. 1. PREVENTION OF EXPERIMENTAL TUBERCULOSIS
WITH INAH AND ITS DERIVATIVES

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A series of experiments was carried out concerning the prophylactic use of isoniazid (INAH) and its derivatives in experimental tuberculosis in guinea pigs.

The results obtained were as follows:

1. A daily dose of 6 mg of INAH per kg of body weight, started simultaneously with challenge infection, suppressed the development of tuberculin skin allergy and the production of antiserum, and was sufficient to prevent development of tuberculosis.

2. The same dose of the drug, started 2 weeks after challenge infection, temporarily reduced the tuberculin skin allergy and antiserum production, but was far less effective in preventing the development of tuberculosis than in the preceding case.

2. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

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No. 3. ON THE EXTRACT OF TUBERCLE BACILLI ISOLATED
FROM PATIENT WITH PULMONARY TUBERCULOSIS

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In experiment I, drug-sensitive human type tubercle bacillus "H₂" (H₂-S), and SM-resistant and INAH-resistant strains derived from H₂-S (SM-R and INAH-R, respectively) were used, and in experiment II, tubercle bacillus isolated from sputum of patients with pulmonary tuberculosis was employed. After cultivation of the bacillus in Sauton media, OT and water extract of the bacilli (P) were pre-

pared in the same way as mentioned in a previous paper.

The OT's and P's thus prepared were examined for their activity in Middlebrook-Dubos reaction and precipitin reaction.

The results obtained were as follows :

Experiment I. The OT from SM-R was inferior in hemosensitizing activity to the OT's from H₂-S and INAH-R, while the P's from the three strains showed no difference in the activity.

Experiment II. (1) The OT's prepared from the strains of various patients differed from one another in hemosensitizing activity and in the activity of inhibiting of Middlebrook-Dubos reaction, while the P's showed almost the same activity.

On the other hand, no difference was observed in the power of causing precipitin reaction between the OT's or between the P's.

(2) No correlation was observed between the drug-sensitivity of the bacillus isolated from the patients and the antigenicity of the OT or P prepared from the bacillus.

(3) The antigenicity of OT and P prepared from each strain did not run parallel.

3. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 15. OT PREPARED FROM CULTURE FILTRATE OF DRUG-
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No. 1. OT FROM SINGLY DRUG-RESISTANT STRAIN

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Four kinds of OT were prepared from human type tubercle bacilli "H₃₇Rv", "H₂", and SM-resistant and INAH-resistant strains derived from "H₂", and the abbreviations, OT-H₃₇Rv, OT-H₂-S, OT-SM-R and OT-INAH-R were employed respectively for them.

The OT's were tested for their activities in Middlebrook-Dubos, precipitin and skin reactions.

The results obtained are summarized as follows :

1. In the activities, the OT's showed the following relationship, OT-SM-R = OT-H₂-S < OT-INAH-R = OT-H₃₇Rv.
2. The same relationship as above was shown by the OT's in their power of conferring antibody-producing activity to erythrocytes by sensitizing.
3. In the activities, the OT's showed only quantitative differences from one ano-

ther but no qualitative ones.

4. Tested by infection to rabbits, H₃₇Rv showed the highest activity for antibody production responsible for Middlebrook-Dubos reaction, and was followed by H₂-S, H₂-SM-R and H₂-INAH-R in that order.

The antibody-producing activity of the bacilli seemed to run parallel to their virulence.

5. Specificity was not observed in the reaction between the antisera of rabbits infected with the four strains and the OT's from the strains.

6. It is of great interest that H₂-INAH-R is inferior to the other three strains in the in vitro production of hemosensitizing and skin-reaction-causing substances.

4. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 15. OT PREPARED FROM CULTURE FILTRATE OF DRUG-
RESISTANT HUMAN TYPE TUBERCLE BACILLI
No. 2. OT FROM DOUBLY DRUG-RESISTANT STRAIN

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The Human type tubercle bacillus, and its SM-resistant and INAH-resistant strains were successively cultured on Ogawa's 1% KH₂PO₄ egg yolk medium containing SM, PAS, INAH, Tbl, VM or o-aminophenol (OM), and singly and doubly drug-resistant strains were isolated.

The original three strains, the isolated strains and H₃₇Rv were cultivated in Sauton medium. After 8 weeks' cultivation OT was prepared from each of the culture filtrates by the conventional method.

The OT's thus prepared were examined for their hemosensitizing, Middlebrook-Dubos reaction inhibiting and precipitating activities.

The results obtained are summarized as follows:

1. In the activities the OT's from INAH- and IHMS-resistant strains were observed to be superior to, but the OT's from the other singly drug-resistant ones to be equally potent as the OT from the original drug-sensitive strain.

2. The strains doubly resistant to SM and INAH derived from different parent strains showed different degrees of activity, the doubly resistant strain yielding OT of almost equal activity as the singly SM-resistant strain or the singly INAH-resistant strain according as whether the former or the latter had been used as parent strain.

3. In each OT, the three activities mentioned above did not always run parallel to the skin reaction causing activity.

5. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 15. OT PREPARED FROM CULTURE FILTRATE OF DRUG-RESISTANT
HUMAN TYPE TUBERCLE BACILLI
No. 3. CLINICAL INVESTIGATION

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Evidence has not been obtained that red cells sensitized with OT from drug-resistant tubercle bacilli are specifically agglutinated or hemolysed by the serum of patient with pulmonary tuberculosis whose tubercle bacillus lost the susceptibility to the drug.

6. STUDIES ON THE INFLUENCE OF CULTURE FILTRATE OF ACID-FAST BACILLI ON THE GROWTH OF VARIOUS BACILLI

PART 1. OBSERVATION ON THE GROWTH OF VARIOUS BACILLI IN THE
MEDIUM CONTAINING CULTURE FILTRATE OF AN ACID-FAST BACILLUS

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The culture filtrate of each of the human type tubercle bacilli "H₂" and "Aoyama B", BOK, avian type tubercle bacillus "Takeo" and Timothy bacillus was added separately to culture media of various bacilli, and their growth in them was observed.

The results obtained were as follows:

1. All the culture filtrates generally promote the growth of enteric bacilli, such as *B. coli communis*, *B. typhi abdominalis*, *B. paratyphi A*, *B. paratyphi B* and *B. dysenteriae* (Komagome B, Shiga, Ohara, Ono and Iishi strains).
2. The culture filtrate of "Aoyama B" remarkably promotes the growth of *staphylococcus* "Terashima" strain and *diplococcus pneumoniae*, but slightly inhibits the growth of *streptococcus hemolyticus*.
3. The culture filtrate of "Aoyama B" has no influence on the growth of anaerobic

bacteria, such as *B. tetani*, *Clostridium welchii* and *Clostridium histolyticum*.

4. The culture filtrate of "Aoyama B" promotes the growth of true fungi, such as *C. albicans*, *C. parakurusei* and *C. pseudotropicalis*.

7. STUDIES ON THE LIVER GLYCOGEN CONTENT OF TUBERCULOUS GUINEA PIGS

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In the present paper, the liver glycogen content of tuberculous guinea pigs was determined over a long period after infection.

The guinea pigs were inoculated subcutaneously in the groin with 0.2 mg of human tubercle bacilli, H₂. Then at intervals of about 10 days, 6 to 10 of these animals were used for one experiment as follows: The fasted animal was anesthetized with ravonal and the liver was removed for the glycogen estimation. Glycogen was precipitated by the method of Good, Kramer and Somogyi, and the reducing sugar in the hydrolysed precipitate was determined by the method of Hagedorn and Jensen.

The results of these analyses revealed that the glycogen content of the liver diminished in tuberculous guinea pigs in comparison with that of the normal control.

Thus, the liver glycogen content of the infected animals showed significant decrease even two weeks after infection, the value decreasing progressively with time, and 40 days or more after infection the mean values of their liver glycogen content were found to be less than 0.2%, while that of the normal controls 2.03%.

It was also observed that the decrease in the liver glycogen content in tuberculous guinea pigs did not parallel the extent of tuberculous lesions in the organ.

8. HISTOCHEMICAL STUDIES ON TUBERCULOUS LESIONS OF THE LUNG

PART 3. HISTOCHEMICAL STUDIES ON TUBERCULOUS
LESIONS OF BCG-SENSITIZED EXPERIMENTAL ANIMALS

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The author performed serological, pathological and histochemical studies of BCG-sensitized guinea pigs which were treated by intravenous and intratracheal inoculation with H₃₇Rv strain of tubercle bacilli.

The results obtained are as follows :

1. The production of antibodies and the allergic condition following the challenge infection were more conspicuous in the BCG-sensitized experimental animals, especially in the intrabronchially inoculated ones, than in non-sensitized animals.

2. The location and severity of the tuberculous lesions in the BCG-sensitized animals did not differ much according to the method of inoculation. The characteristics of the lesions resembled those of intravenous inoculation in non-sensitized animals.

3. Ribonucleic acid was demonstrated in the small round cells and the epitheloid cells of fresh tuberculous lesions in the lung and the liver of the BCG-sensitized animals where cellular activity was vividly taking place, at the early stage (2-4 weeks after the inoculation), the amount decreasing later (5 months after the inoculation). However, the substance was seen in the epitheloid cells of the tuberculous lesions of the spleen throughout the entire period from the early stage to the later stage.

4. Vigorous activity of some enzymes, such as alkaline phosphatase, alkaline ribonucleophosphatase, acid ribonucleophosphatase, acid desoxyribonucleophosphatase, alkaline desoxyribonucleophosphatase and 5-nucleotidase was seen in the tissue of fresh tuberculous lesions of the BCG-sensitized animals, while the activity of lipase, acid phosphatase and adenosintriphosphatase was found to be slack.

In particular, the activity of alkaline phosphatase was seen to be especially vigorous in the tissue of tuberculous lesions produced by intratracheal and intravenous inoculation.

5. Polysaccharides were found in the experimental lesions of the BCG-sensitized animals, in relative abundance in the small round culls, the epitheloid cells, the caseous tissue and the connective tissue surrounding it.

6. Fat and lipid were observed in the fresh experimentally produced tuberculous lesions of the BCG-sensitized animals. The reaction for fat was seen to increase one month after the inoculation when caseation became conspicuous in the lesions.

9. A CASE REPORT OF LIGATION OF PULMONARY ARTERY FOR TUBERCULOUS PULMONARY LESION

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The Left major pulmonary artery was accidentally ligated, when resection of the upper lobe was carried out in pulmonary tuberculosis.

Five months after the operation, the residual lesion of the left lung became aggravated and resulted in cavity formation accompanied by presence of *Mycobacterium* in the sputum.

It seemed that the aggravation of the lesion was due both to the ligation of pulmonary artery and to the appearance of a resistant strain in the lesion.

10. FURTHER OBSERVATIONS ON THE STREPTOLYSIN-S SUSCEPTIBILITY OF TANNIC ACID-TREATED ERYTHROCYTES

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Following the observation that erythrocytes of certain species of mammals, when treated with suitable concentration of tannic acid (TA) in the cold, lose the susceptibility to the hemolytic action of streptolysin S (St-S), an attempt was made to ascertain whether or not the toxin was really ineffective on the TA-treated red blood cells.

The erythrocytes of ox and goat were used.

The streptolysin S used was the purified sample prepared from 1% ribonucleic acid-broth cultures of hemolytic streptococci (minimum hemolytic concentration = 1:10,000,000), 2.5 ml of its 1:100,000 solution being theoretically sufficient to lyse completely 0.5 ml of 50% red cell suspension at 37°C in 2 hours.

The preparation of TA-treated erythrocytes was carried out in the same way as described previously. One part of the washed red cells (50% suspension) was treated with ten parts of a 1:5,000 or 1:10,000 solution of tannic acid in an ice-water bath for 10 minutes, and the cells were then washed 3 times in cold buffered saline. The treated erythrocytes were incubated, under various conditions, with an amount of St-S. At the end of incubation, the mixtures were centrifuged, and the hemolytic activity of the supernatant obtained was tested on untreated (normal) erythrocytes, and compared with the activity of control solutions of St-S kept under the same experimental conditions.

The results of the experiments are summed up as follows:

1) When 0.5 ml 50% goat erythrocyte suspension, after treatment with TA, was incubated with 2.5 ml of a 1:100,000 solution of St-S at 37°C, the hemolytic activity of the supernatant decreased rapidly until practically none was demonstrable at the end of 60 minutes' incubation. The rate of loss of the activity decreased with

lowering of the temperature at which the red cells were incubated with St-S, no loss of the activity occurring at an ice-water temperature even after 2 hours.

2) It was shown in the experiments with ox erythrocyte that the loss of the hemolytic activity was complete when St-S was incubated with an equivalent or excess amount of erythrocyte, while it was incomplete in the range of toxin excess.

3) By selecting appropriate conditions, it was possible to demonstrate that the TA-treated erythrocytes, after incubation with suitable concentration of St-S and thorough washing, lysed upon standing at 37°C. This hemolysis of the TA-treated erythrocytes could not be inhibited by trypan blue which is strongly inhibitory against streptolysin hemolysis of normal erythrocytes.

11. STUDIES ON THE STREPTOLYSIN-S SUSCEPTIBILITY OF ENZYME-TREATED ERYTHROCYTES

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Washed red blood cells of the rabbit were treated with various enzymes under conditions optimal for the respective enzymes, and then their susceptibility to streptolysin S was studied in comparison with that of normal cells.

Commercial preparations of the following enzymes were used:

- 1) pepsin, trypsin, papain, subtilisin, and erepsin.
- 2) lipase.
- 3) alpha- and beta-amylase, beta-glucosidase, hemicellulase, and hyaluronidase.

It was shown that the enzyme-treated red cells did not differ, in any way, from untreated normal cells in their sensitivity to the lysin.

12. STUDIES ON THE EFFECT OF PROTEINS UPON THE STREPTOLYSIN-S SUSCEPTIBILITY OF TANNED ERYTHROCYTES

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The present paper concerns the restoration by proteins of the lost susceptibility

to streptolysin S of tannic acid-treated erythrocytes.

Erythrocytes of guinea pig were used. The red cells pretreated with a solution (1:10,000) of tannic acid were suspended in a solution of the substance to be tested and incubated at 37°C for 20 minutes. At the end of incubation, the cells were washed and resuspended in buffered saline in a concentration of one per cent. The red cell suspensions thus obtained were assayed for their susceptibility to streptolysin S.

It was shown that the reduced susceptibility of the tanned erythrocytes to streptolysin S was readily restored by treating them with certain proteins. Of the proteins studied, gelatin was found to be the most effective; the tanned red cells incubated in 1:20,000–50,000 solutions of gelatin were shown to be equally sensitive to streptolysin S as normal red cells, and gelatin, even in a concentration of 1:200,000, was sufficiently effective to produce noticeable restoration of the reduced susceptibility. Albumin and γ -globulin were also effective, but far less so than gelatin. Protamin, in the range of tested concentrations, was inactive. Peptone and casein hydrolysate showed little or no activity. Among the other substances tested, including nucleic acids and polysaccharides, the 2 mucopolysaccharides, chondroitin sulfate and mucin, showed a considerable effect to restore the streptolysin-S sensitivity of the tanned red cells.

13. IMMUNOCHEMICAL ANALYSIS ON THE BLOOD TRANSFUSION MATERIALS

PART I. ON THE PLASMANATE

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Human serum albumin, Plasmanate and human dried plasma were analysed on their protein components immunochemically and moreover, the common factors of monkey, bovine and human serum were investigated.

Namely, the homologous antisera against each materials were prepared by Freund's adjuvant method (double intramuscular injection). Then, by means of agar gel double diffusion technique and immunoelectrophoresis, antigens of these materials were showed as the patterns of precipitin line spectrum and compared with that of normal human serum.

The result obtained were as follows;

1. On human serum albumin and Plasmanate, the heating at 60°C, 10 hours for the inactivation of hepatitis-virus showed neither any change in their antigenicities nor any artificial components which supposed to occur in this procedure, if the heating is strictly not over 60°C.

2. By both the lyophilization and the alcohol fractionation (Cohn), most antigens of human plasma did not change their antigenic properties, although quantity of a few antigens showed a slight decrease.

3. Both monkey and bovine serum had some antigens to be able to precipitate with albumin fraction of human serum, but common factors a little in monkey and almost heterologous in bovine.

Publications not appearing in the Ann. Rep. Tbc. Kanazawa (1959)

- 1) **Koshimura, S., Murasawa, K., Hirata, R. and Bando, Y. :** Further Experiments on the Carcinostatic Action of Bis(2-hydroxy-3,5-dibromophenylazo)-n-propylphloroglucinol
 A brief summary of the experimental data so far obtained in the study of anticancer activity of 2,2'-dihydroxyazobenzene derivatives was attempted. It is particularly notable that Azo-106 was proved to be very effective against Yoshida sarcoma, Ehrlich carcinoma and Sarcoma 180 in vivo.
ACTA, 15, 154, 1959.

- 2) **Miyaji, T. :** Experimental Anticancer Studies. Part 9
 Test for Tumor-inhibitory Action of Bis (2-hydroxy-3,5-dibromophenylazo)-n-propylphloroglucinol (Azo-106) Administered in a Part of Body Remote from the Site of Tumor Implantation in Mice (Japaneses)
 Using Ehrlich ascites carcinoma, and Sarcoma 180, as the implantation material to mice, following series of anticancer experiments were designed :
 1. Experiments on animals implanted with tumor cells intraperitoneally.
 - a) Effect of subcutaneous administration of Azo-106 on the life-span of the animals.
 - b) Effect of oral administration of Azo-106 on the life-span of the animals.
 2. Experiments on animals implanted with tumor cells subcutaneously in the left groin.
 - a) Effect of intraperitoneal administration of Azo-106 on the growth of tumor.
 - b) Effect of subcutaneous administration of Azo-106 on the growth of tumor.
 Summing up the results obtained in all these experiments, it was concluded that Azo-106, even when it is administered at a place distant from the tumor, exerts an inhibitory activity against the tumors.
Juzen-igakkai-zasshi, 63, 278, 1959.

- 3) **Shoin, S. :** Experimental Anticancer Studies. Part 10
 On the Effect of Avirulent-mutant Strain of *Streptococcus hemolyticus* upon Invasion Power of Ehrlich Ascites Carcinoma in Mice
 Data was presented to show that when Ehrlich carcinoma cells were contacted in vitro with a nearly avirulent hemolytic streptococci for a short time, there occurred a lose of the invasion power of the carcinoma cells to mice.
 The present anticancer experiment differs from that reported previously in that penicillin was not employed.
Japan. J. Exp. Med., 29, 529, 1959.

- 4) **Miyaji, T., Kirita, T., Ohtsuki, H. and Kadono, K.** : Experimental Anti-cancer Studies. Part 11

Influence of Heating on the Ability of Hemolytic Streptococci to Injure Cancer Cells (Japanese)

Data were presented to show that when hemolytic streptococci, suspended in phosphate-buffered Ringer solution (pH 7.2), were heated for 30 minutes at temperature higher than 45°C, there occurred a complete loss in their ability to injure the invasion power of Ehrlich carcinoma cells to mice.

Juzen-igakkai-zasshi, 63, 286, 1959.

- 5) **Miyaji, T.** : Studies on the Phenomenon of High Promotion by Nucleic Acid of the Production of Streptolysin S of Hemolytic Streptococcus. Part 18

Influence of Heating, Ultraviolet Irradiation and Bacterial-body Disruption on the Streptolysin S Formation Ability of *Streptococcus hemolyticus* (Japanese)

Data were presented to show that the streptolysin S formation ability of *Streptococcus hemolyticus* was affected either by heating of cocci, or by exposure of cocci to ultraviolet ray, or by mechanical disrupting procedure of bacterial body.

Juzen-igakkai-zasshi, 63, 291, 1959.