

# ABSTRACTS

## 1. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 10. METHODS OF IMMUNIZING RABBITS AGAINST TUBERCULOSIS EVALUATED BY SERUM REACTION

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In the present study, the following four methods of immunizing rabbits were compared as to the power of the animal serum responsible for Middlebrook-Dubos reaction and for precipitin reaction. As antigen was used the extract of human tubercle bacillus "H<sub>2</sub>" strain prepared by boiling for an hour in neutral distilled water.

- 1) One single intravenous injection of 1 mg of living human tubercle bacilli suspended in 0.2ml of physiologic saline.
- 2) One single subcutaneous injection of 25 mg of heat-killed human tubercle bacilli suspended in 1 ml of paraffin oil.
- 3) Several weekly intravenous injections of 10~15 mg of heat-killed human tubercle bacilli suspended in 2~3ml of physiologic saline.
- 4) One single subcutaneous injection of 25 mg of BCG suspended in 1 ml of physiologic saline.

The data presented show that the titers were significantly higher in the rabbits receiving weekly intravenous injections of heat-killed tubercle bacilli.

## 2. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 11. PRECIPITINOGEN CONTENT OF AQUEOUS EXTRACTS OF ACID-FAST BACILLI NO. 1. EVALUATION OF EXTRACTION METHODS

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Attempts were made to obtain specific precipitinogen, if any, from heat-killed human tubercle bacillus "H<sub>2</sub>" strain to immune serum of rabbit treated with the bacillus. For this purpose, bacillary body extracts were obtained under various extraction conditions from various acid-fast bacilli such as human tubercle bacilli "H<sub>2</sub>" and "Aoyama B" strains, BCG, bovine and avian tubercle bacilli and two non-pathogenic bacilli, timothy bacillus and smegma bacillus, and the precipitin titers of the serum shown by the extract of the bacilli obtained

under an identical condition were compared. The results obtained are summarized as follows:

1) The precipitin titer of the serum as measured by the extract of tubercle bacilli showed significant differences according to their types, but only when the extract was prepared with distilled water acidified to pH 3.2 with HCl.

2) The highest precipitin titer of the serum was given when measured with the extract prepared with neutral distilled water, no differences being observed when extracts of different bacilli were used. The titer was lower when extracts prepared with acidified (HCl to pH 3.2) water were used, but in this case the human type bacillus yield an extract giving higher titer than the others. Furthermore, when the extracts were prepared with physiologic saline acidified with HCl, the titer was highest when the pH was 3.2 and lowest when the pH was 4.6.

### 3. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

#### PART 11. PRECIPITINOGEN CONTENT OF AQUEOUS EXTRACTS OF ACID-FAST BACILLI No. 2. CROSS PRECIPITIN TESTS

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In the preceding study, the author observed that the extract of human type tubercle bacilli in distilled water acidified to pH 3.2 with HCl reacted in the precipitin test with the serum of rabbits immunized with the human type bacilli "H<sub>2</sub>" strain more strongly than extracts of tubercle bacilli of other types prepared in the same manner.

In the present study, attempts were made to ascertain if extracts of human, bovine and avian tubercle bacilli reacted specifically in the precipitin test with the serum of rabbits immunized with bacilli of the same type.

The results obtained are summarized as follows :

1) The precipitin titer of each serum measured by extracts of bovine and avian tubercle bacilli in distilled water was much lower when the water was acidified to pH 3.2 with HCl.

But no such difference due to acidity of the extracting water was observed when human type bacilli were used.

2) For all the three types of bacilli the acid extracts were much poorer than the neutral extracts in the antigenicity for highly diluted sera.

3) The precipitin titer of every serum suffered conspicuous lowering by absorption by bacilli of different types as well as the same type.

## 4. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 11. PRECIPITINOGEN CONTENT OF AQUEOUS  
EXTRACTS OF ACID-FAST BACILLINo. 3. PRECIPITIN REACTION WITH THE POLYSACCHARIDES AND  
THE PROTEINS ISOLATED FROM THE AQUEOUS EXTRACTS

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In the present study, protein and polysaccharide fractions were prepared from the extract of defatted human tubercle bacilli "H<sub>2</sub>" stain in neutral distilled water and the difference in antigenicity of the fractions was observed before and after hydrolysis either with HCl or with NaOH.

For this purpose, the precipitin reactions with the ring test between the antiserum of rabbits treated with heat-killed human tubercle bacilli "H<sub>2</sub>" strain and the fractions, and between the antiserum and the hydrolysates of the fractions, were observed as well as the forms of "the fields of reaction". The hydrolysis was effected by adjusting the solution of the fractions either with HCl to PH 3.2 or with NaOH to PH 11.0, and then heating it at 100°C for 2 hours. The results obtained are summarized as follows.

1) "The field of reaction" in the precipitin reaction between the polysaccharide fraction and the antiserum was observed to consist at least of three unitary reaction systems. "The field of reaction" was observed to reduce in size remarkably when the HCl-hydrolysate was used instead of the undecomposed polysaccharides, whereas only "the field of reaction" of highly diluted antibody unitary system disappeared when the NaOH-hydrolysate was used.

2) "The field of reaction" between the protein fraction and the antiserum was observed to consist apparently of a single unitary reaction system, and "the field of reaction" remained unchanged in size even when the HCl- or NaOH-hydrolysate was used instead of the undecomposed proteins.

## 5. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 12. ACTIVITY OF THE BACILLARY BODY EXTRACT PREPARED  
UNDER VARIOUS CONDITION AS HEMOSENSITIN

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Observation was made of the hemagglutination reaction between sera of rabbits immunized with human, bovine and avian type tubercle bacilli and red blood cells sensitized with

extracts of the three types of bacilli prepared with hot distilled water, adjusted either neutral or to pH 3.2 with HCl or to pH 11.0 with NaOH. No specificity of the reaction was noticed.

## 6. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 13. STUDIES ON CELL EXUDATION IN THE PERITONEAL CAVITY

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Examination of cell exudation in the peritoneal cavity was carried out, after injection of 4 substances.....liquid paraffin, physiological saline and dead tubercle bacilli suspended in these 2 substances.....into the peritoneal cavity of normal guinea pigs and guinea pigs sensitized with liquid paraffin vaccine. The results obtained were as follows.

1) Polymorphonuclear cells were the main component in the earlier stage of cell exudation in the peritoneal cavity, but were gradually replaced by mononuclear cells in the later stage.

2) Polymorphonuclear cells were found to keep exuding for a long time and mononuclear cells were observed to be late in appearance when dead bacilli suspended in liquid paraffin or physiological saline were injected.

3) Quicker replacement of polymorphonuclear cells with mononuclear cells in the peritoneal cell exudation was observed in sensitized guinea pigs, than in normal guinea pigs, whichever of the four substances were injected.

4) The phagocytic activity of the exuding cells against the dead tubercle bacilli injected into the peritoneal cavity, was observed stronger in sensitized guinea pigs than in normal guinea pig.

Judging from double phagocytic activity of the mononuclear cells, they may be regarded the agents ultimately disposing of the dead bacilli.

## 7. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 14. STUDIES ON PHAGOCYtic ABILITY OF PERITONEAL EXUDATE CELL AND INFLUENCE OF TUBERCULIN UPON THE ABILITY

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Examination was carried out concerning the phagocytic activity of peritoneal exudate cells of tuberculin sensitive animal (washed and resuspended in physiological saline, that is,

free from the so called humoral factor) the results were as follows.

a) Stronger phagocytic activity was observed in the cells from guinea pigs sensitized with killed bacilli suspended in liquid paraffin than in those normal guinea pigs, and most of the phagocyte cells were mononuclear.

b) Marked decline of phagocytic ability was caused in the cells from sensitized guinea pigs by the addition of old tuberculin to the cell suspension.

## 8. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 15. STUDIES ON PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY NO. 1. EXPERIMENTS WITH PERITONEAL EXUDATÉ CELLS, VISCERAL CELLS AND SERUM FROM GUINEA PIGS SENSITIZED WITH DEAD BACILLI SUSPENDED IN LIQUID PARAFFIN

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1) Tuberculin sensitivity was passively transferred to normal guinea pigs by means of peritoneal exudate cells from donor guinea pigs sensitized with killed bacilli suspended in liquid paraffin.

But the sensitivity was not successfully transferred with serum from donor guinea pigs.

2) Tuberculin sensitivity transferred passively was observed to last only about 72 hours.

3) Both polymorphonuclear cells in the peritoneal exudate from the donor guinea pigs were found to have the ability to transfer the tuberculin sensitivity to normal recipient guinea pigs.

4) Reverse transfer as reported by Metaxas could not be demonstrated.

5) Passive transfer of tuberculin sensitivity was not effected with cells killed by heat or cold.

6) Tuberculin sensitivity was also passively induced in normal guinea pigs by means of suspension of tissue cells from the spleen, liver and lung of the sensitized guinea pig, but not from the brain.

## 9. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 15. STUDIES ON PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY NO. 2. SEARCH FOR CIRCULATING ANTIBODIES IN TUBERCULIN SENSITIVITY PASSIVELY TRANSFERRED GUINEA PIGS

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Positive conversion of tuberculin skin reaction and Schultz-Dale reaction was observed to occur in guinea pigs injected with peritoneal exudate cells from tuberculin positive guinea pigs sensitized with dead bacilli suspended in liquid paraffin, but no antibody responsible for Middlebrook-Dubos reaction or Inoue's tubercle bacilli agglutination reaction was found in the serum of recipient guinea pigs, nor any rise of phagocytic ability of the leucocyte was noticed.

## 10. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

### PART 15. STUDIES ON PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY NO. 3. CROSS SKIN SENSITIVITY TEST IN GUINEA PIGS INJECTED WITH PERITONEAL EXUDATE CELLS AND SERUM FROM GUINEA PIGS SENSITIZED WITH EGG ALBUMIN

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Intradermal tests with egg albumin and old tuberculin were carried out on the recipient guinea pigs, 24 hours after injection of peritoneal exudate cells and serum from donor guinea pigs sensitized with egg albumin, and the results were as follows:

- a) Distinct reaction was noticed when the test was performed with egg albumin, whether the cells or the serum had been injected, and
- b) The reaction was negative when the test was performed with old tuberculin in all cases.

That is, the peritoneal exudate cells, as well as the serum from guinea pigs sensitized with egg albumin was not capable of effecting passive transfer of tuberculin sensitivity to normal guinea pigs, demonstrating the specificity of the passive transfer of egg albumin sensitivity.

## 11. STUDIES ON RESISTANCE OF MICROORGANISM TO VARIOUS CHEMICALS

### PART 8. MECHANISM OF ACQUISITION AND LOSS OF DRUG RESISTANCE BY BACTERIA NO. 1. EXPERIMENTS WITH AVIAN TYPE TUBERCLE BACILLI

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Experiments were made for the purpose of clarifying the relation between the permea-

bility of the cell membrane and the streptomycin-resistance of the avian tubercle bacillus.

#### Experiment I.

A strain of the avian tubercle bacillus, demonstrated not to grow on 1% Ogawa's egg medium containing more than 17 per ml of streptomycin (SM), was cultivated successively both on 6% and 20% glycerol media.

The abbreviated nomenclature, 6% (20%) glycerol medium, is employed to denote Sauton's synthetic medium, in which the glycerol content is 6% (20%) and the nomenclature, 6% (20%) GS, to denote the avian tubercle bacillus grown on 6% (20%) glycerol medium. It was found that 20% GS was observed to show (1) stronger resistance to decolorization by boiling, (2) longer lag phase in  $O_2$ -uptake measured by Warburg's method than the 6% GS and (3) to have the growth inhibited only when the concentration of SM in the medium exceeded 107 per ml.

#### Experiment II.

A strain of the avian tubercle bacillus resistant to 1,0007 per ml of SM was suspended in bacillary body extract of a SM-sensitive strain of avian tubercle bacilli.

When the suspension was heated at 50°C for 30 minutes, then frozen at -13°C for 20 minutes and finally thawed, it was found that the bacillus absorbed a larger quantity of  $P^{32}$ , contained in the culture medium (add in the form of  $Na_2HP^{32}O_4$ ) and had lower SM-resistance than when the suspension was left at room temperature all the while.

The data obtained from the experiments I and II suggest that SM-resistance should have some correlation with the permeability of the bacterial cell membrane.

## 12. A STUDY ON THE TUBERCULIN-PRODUCING PROPERTY OF ATYPICAL ACID-FAST, CHROMOGENIC MYCOBACTERIUM, ISHII STRAIN, WITH SPECIAL REFERENCE TO THE CITRATE-TUBERCULIN PRODUCTION

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In the present study an atypical acid-fast, chromogenic mycobacterium, Ishii strain (No. 22) which was kindly supplied by The National Institute of Health of Japan, was tested for the tuberculin-producing property in comparison with *M. tuberculosis* var. *hominis* H<sub>2</sub> strain.

A series of flasks each containing 50 ml of Sauton's medium were inoculated with Ishii strain and another similar series, with H<sub>2</sub> strain, and both were incubated at 37°C.

Three flasks were taken out of each series after periods of incubation of 5, 7 and 9 weeks respectively and treated as follows :

The culture fluid was separated from the bacilli and passed through a Seitz filter (Culture-Filtrate (CF)). The bacilli were washed, immersed in 0.1 M citrate and incubated at 37°C. for 24 hours, at the end of which time the citrate solution was passed through a Seitz



filter (Citrato-Tuberculin (CIT)). The tuberculin potencies of CIT and CF samples thus obtained from Ishii strain were compared with the potencies of the corresponding samples from H<sub>2</sub> strain upon tuberculous guinea pigs infected with H<sub>2</sub>.

The comparative skin tests showed that all the tuberculin samples from Ishii strain, both CIT and CF, were much less potent than those from H<sub>2</sub> strain.

### 13. HISTOLOGICAL STUDIES ON TUBERCULIN ALLERGY

#### PART 3. THE EFFECTS OF VARIOUS DRUGS UPON TUBERCULIN REACTION NO. 1. EXPERIMENTS WITH OLD TUBERCULIN

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For the purpose of clarifying the mechanism of tuberculin reaction, experiments were made to study the influence upon the reaction exerted by some drugs affecting the permeability of capillary vessels and others acting on peripheral autonomous nerves. For this purpose, the tuberculin skin test was carried out by old tuberculin (OT) mixed with the drugs firstly on human beings having natural infection and secondly on rabbits sensitized with paraffin oil heat-killed human tubercle bacilli "H<sub>37</sub>Rv".

The concentration of each drug used and its effect upon tuberculin reaction in human beings are shown in table 1.

A) The power of OT to produce skin reaction in human beings was weakened by the addition of epirenamine hydrochloride,  $\beta$ -dimethylaminoethylbenzhydrylether,  $\beta$ -imidazolaminoethyl or cortisone, and strengthened by rutin, adrenochrommonosemicarbazone or hyaluronidase, but was not affected by atropine sulfate, acetylcholine, ergotamine, vitamin C, vitamin K or vitamin P. B. The power of OT to produce skin reaction in rabbits was, as observed in human beings, weakened by the addition of epirenamine hydrochloride,  $\beta$ -dimethylaminoethylbenzhydrylether,  $\beta$ -imidazolaminoethyl or cortisone, and strengthened by hyaluronidase.

The histological findings of the local skin reaction of the rabbits at 6, 12, 24, 48 and 72 hours after injection are summarized as follows:

1) In general, both the polymorphonuclear cell infiltration at the later stage caused by OT were diminished by the addition of epirenamine hydrochloride,  $\beta$ -dimethylaminoethylbenzhydrylether,  $\beta$ -imidazolaminoethyl or cortisone while the addition of hyaluronidase caused no change in the degree of the cell infiltration except slight decrease of mononuclear cell infiltration at 12 hours after injection.

2) At the early stage, the blood vessel reaction caused by OT was intensified by the addition of epirenamine hydrochloride, cortisone or hyaluronidase, and reduced by  $\beta$ -dimethylaminoethylbenzhydrylether, but was not affected by  $\beta$ -imidazolaminoethyl.

Table 1

Agents added to OT	Concentrations of agents in 0.1ml of OT diluted in 1:100 or 1:1,000	Effect
Atropine sulfate	0.025mg	0
Acetylcholine	0.025mg	0
Eprenamine hydrochloride	0.05 mg	—
Ergotamine	0.05 mg	0
Vitamin C	1.0 mg	0
Vitamin K	0.5 mg	0
Vitamin P	1.25 mg	—
Rutin	1.0 mg	+
Adrenochrommonosemicarbazone	0.125mg	+
Hyaluronidase	1.0T.R.	+
$\beta$ -imidazolethylamine	0.5 $\gamma$	—
$\beta$ -dimethylaminoethylbenzhydylether	0.5 mg	—
Cortisone	0.625mg	—

+ : Promoting effect  
 — : Inhibiting effect  
 0 : Without influence

**Publications not appearing in the Ann. Rep. Tbc.,  
Kanazawa (1957).**

- 1) **Kato, S. :** Inactivation of Tuberculin by Iodine. Japan. J. Tuberc., 4 (2-4), 119, 1956.

Data were presented to show that the biological activity of tuberculin, including Old Tuberculin, purified citrate-tuberculin and o-aminophenol azo-tuberculin derivatives, was readily inactivated by iodine in neutral or alkaline medium. The rate of inactivation by iodine increased either with increase in temperature or with decrease in hydrogen ion concentration. The iodine effect upon tuberculin was inhibited by the presence of other non-specific proteins.

Some discussions were made concerning the possibilities responsible for the iodine inactivation of tuberculin.

- 2) **Yoshimura, M. :** Studies on the Influence of Nitrous Acid upon the Immunogenic Properties of Tubercle Bacilli. Japan. J. Tuberc., 4 (2-4), 145, 1956.

It was shown that nitrous acid, when added to the living cell suspension of a virulent human tubercle bacillus, strain H<sub>2</sub>, at low temperature in acetate buffer medium (pH 4.1-4.2), rendered the organisms nonviable within a short time.

Guinea pigs receiving these nitrous acid-treated vaccines by the route of intraperitoneal administration developed a high level of resistance to subsequent infection with living H<sub>2</sub>, the level of which was at least equal to that produced in guinea pigs vaccinated with BCG and superior to that in animals vaccinated with heat-killed H<sub>2</sub>.

- 3) **Kigoshi, S. :** On the Tuberculin-Hypoglycemia in Tuberculous Guinea Pigs. Japan. J. Tuberc., 4 (2-4), 153, 1956.

Blood sugar determination experiments on tuberculous guinea pigs receiving a lethal dose of tuberculin revealed that severe and prolonged hypoglycemia occurred in all these animals.

Some discussions were held on the possible mechanism of the hypoglycemia-causing action of tuberculin.

- 4) **Shimizu, S. :** Formation of Tuberculin by Washed Tubercle Bacilli in Citrate Solution. Part IX. Comparative Study on Some Biological Properties of Streptomycin-Resistant and Streptomycin-Sensitive Cells of Various Strains of *Mycobacterium Tuberculosis* with Special Reference to Tuberculin Production. Japan. J. Tuberc., 5 (1-2), 35, 1957.

Streptomycin-resistant mutants were produced *in vitro* from a variety of strain of human and bovine type of *M. tuberculosis*. They were tested for their tuberculin-producing property in comparison with their parent drug-sensitive strains. It was found that among the streptomycin-resistant mutants the one derived from human tubercle bacillus, *Aoyama B* strain, possessed a precipitously reduced ability to produce tuberculin-active substance(s) in a citrate solution, and in addition, that "citrate-tuberculin" solution thus obtained from this variant contained no detectable free amino acids in contrast with the "citrate-tuberculin" solutions obtained from other strains studied.

Some discussions were made on the possible correlation between the development of streptomycin resistance of tubercle bacilli and the biological and biochemical characteristics observed with the drug-resistant variant.

- 5) **Ishida, S.** : Formation of Tuberculin by Washed Tubercle Bacilli in Citrate Solution. Part XI. Study on the Physical and Chemical Properties of the Purified "Citrate-Tuberculin", with Special Reference to its Biological Activity. *Japan. J. Tuberc.*, 5 (1-2), 61, 1957.

The effects of various physical and chemical agents on the highly potent purified tuberculin, a protein fraction obtained from "citrate-tuberculin", were studied with the following results :

1. The purified tuberculin, in neutral, was very resistant to high temperature (up to 100° for hours).
2. The purified tuberculin was much more sensitive to alkali than to acid.
3. The purified tuberculin did not appreciably dialyze through the cellophane membrane.
4. Prolonged irradiation with ultraviolet light caused partial inactivation of the purified tuberculin.
5. Of proteolytic enzymes tested, pepsin, trypsin and papain effectively inactivated the purified tuberculin, but erepsin failed to cause a significant degree of inactivation of the toxin.

The lipase preparation used were found to be able to inactivate the purified tuberculin at a rate comparable with that of the proteolytic enzymes.

Various carbohydrate-splitting enzymes produced no effect on the purified tuberculin.

The purified tuberculin was unimpaired by the action of snake venom, ribonuclease and urease.

6. The purified tuberculin, under the conditions employed, was fairly resistant to the action of a number of chemical reagents, that is, the various oxidizing and reducing reagents as  $H_2O_2$ , ferricyanide, oxygen,  $KMnO_4$ ,  $Na_2S$ , thioglycolate, cystein, ascorbate and 1-amino-2-naphthol-4-sulfonate ; the sulfhydryl reagents as monoiodoacetate and p-chloromercuribenzoate ;  $HNO_2$  ; formaldehyde ; dinitrofluorobenzene ;  $POCl_3$  ; dimethyl sulfate. On the contrary, the purified tuberculin was found to be readily inactivated by treatment with benzoyl chloride.

- 6) **Umezaki, S., Nishida, S. and Tsunemoto, H.** : Comparative Experiment of Potency in Skin Reaction between o-Aminophenol Azo-Tuberculin and Six OT Preparations in Japan. *Kokyuki-shinryo*, 12 (5), 405, 1957.

In measuring the potency of six OT preparations, all well-known and widely used in Japan, employing as standard of comparison, o-aminophenol azo-tuberculin prepared in the Research Institute of Tuberculosis in Kanazawa University, it was revealed that one preparation was of higher, another of lower and three others of equal activity as the standard. The behavior of the remaining one preparation toward human subjects indicated that it was totally different in nature from the standard and no comparison of the potency could be legitimately be made.

- 7) **Saegusa, K.** : Studies on Resistance of Microorganism to Various Chemicals. Part 7. Experiments on a Human Tubercle Bacillus "Yonetani Strain" Isolated from a Pulmonary Tuberculosis Patient and Resistant to SM, PAS and INAH. *Tokyo Med.*

J., 74 (12), 21, 1957.

A strain of human tubercle bacillus, highly resistant to SM and resistant to a lower degree to PAS and to INAH was isolated from a pulmonary tuberculosis patient receiving SM, PAS, INAH and o-aminophenol (OM). The strain was injected into mice, and the virulence of the strain and the therapeutic effect of antituberculous agents such as SM, PAS, INAH, OM and PZA were examined. The results obtained were as follows :

1) The strain produced almost equal grade of the lesions in visceral organs as H<sub>2</sub> strain.

2) All the agents used proved to be effective for the mice infected with the strain, but SM was observed to be less effective than the others.

3) By means of successive passage of the strain through mice, the SM-resistance was not observed to change, while the PAS- and INAH-resistance were observed to decrease gradually.

- 8) **Shimizu, R. :** A Study on the Regeneration of Ribonucleic Acid from Ag-RNA-Complex by Desilvering Procedure. II. J. Pharm. Soc., Japan, 77 (6), 676, 1957.

An examination was made for the process of regenerating ribonucleic acid from its complex with silver, reported in the preceding paper, and following processes were carried out.

1) To 100 ml of 1 % aqueous solution of silver-ribonucleic acid complex, 1 ml of 5 % ammonia water and 20 ml of saturated sodium chloride solution are added in that order, and hydrogen sulfide is passed through this solution to precipitate the silver.

2) This solution is then centrifuged, to its supernatant solution is added ethanol, the precipitate formed is collected. The precipitate is dissolved in distilled water, dialyzed, and ethanol added to the dialyzate in the membrane. The precipitate thereby obtained is washed with ethanol and ether, and dried under a reduced pressure.

By such a procedure, white amorphous powder is obtained that agrees with ribonucleic acid in various physical and chemical properties as well as in biological activity, such as the promotion of streptolysin S production by *Streptococcus hemolyticus*, and entirely devoid of silver. This regenerated ribonucleic acid is obtained in 80-90% yield and its purity was found to be far higher than the commercial yeast ribonucleic acid preparation by the ninhydrin reaction and sulfur detection tests.

- 9) **Shimizu, R. :** A Method for Purification of Ribonucleic Acid through the Formation of Ag-RNA-Complex. III. J. Pharm. Soc., Japan, 77 (6), 561, 1957.

The fact that the ribonucleic acid regenerated from its silver complex is of higher purity than that of commercial yeast ribonucleic acid preparation suggests the possibility of the use of this process for purification of ribonucleic acid preparations. The commercial preparation of sodium yeast ribonucleate was found by ninhydrin reaction tests to be contaminated with a fair amount of proteinic substances. Therefore, such a commercial preparation was submitted to comparative tests of deproteinization by purification through silver-ribonucleic acid complex formation and by Sevag's chloroform gel method. The former was found to be highly effective, the deproteinization effect obtained finally by nine repetition of the chloroform gel process being attained in one purification through the silver-ribonucleic acid complex.

It was assumed that the deproteinization of this purification through the silver complex is effected by colloidal absorption of occluded impurities by the silver sulfide

formed. Therefore, copper nitrate or nickel nitrate and hydrogen sulfide can also effect purification of nucleic acid but the yield in such a case is only around 20-40% against that of 80-90% by the silver complex method. Further, the purification process becomes more complicated by the use of copper or nickel salts.

- 10) **Shimizu, R., Himeno, Y., Kishi, G. and Ito, T. :** Studies on the Phenomenon of High Promotion by Nucleic Acid of the Production of Streptolysin S of Hemolytic Streptococcus. Part 17. Study on the Influence of Methyl Ribonucleic Acid upon the Streptolysin S Formation of Hemolytic Streptococci. *Juzen-igakkai-zasshi*, **59** (2), 127, 1957.

Yeast ribonucleic acid was methylated with methyl iodide according to the method described by Anderson et al. first, and the product thus obtained was tested for its influence on the production of Streptolysin S. As a result, no positive evidence was obtained by the experiment with methylated polynucleotide in promoting streptolysin S production.

- 11) **Hirata, R. :** Experimental Anticancer Studies. Part 5. Bis (2-hydroxy-3,5-dibromophenylazo)-alkylphloroglucinols and Their Inhibitory Effect Against Ehrlich Ascites Carcinoma in Mice. *Japan. J. Exp. Med.*, **27**, 99, 1957.

For the purpose of extending the study on the carcinostatic action of 2,2'-dihydroxyazobenzene derivatives, five homologues of bis (2-hydroxy-3,5-dibromophenylazo)-alkylphloroglucinol, namely ethyl-, n-propyl-, n-amyl-, n-hexyl- and iso-hexyl- derivatives, were newly synthesized and tested for their inhibitory effect against Ehrlich ascites carcinoma in mice.

The result of such anti-tumor experiments revealed that all the five azo-alkylphloroglucinol derivatives were effective in causing the inhibition of the tumor growth in mice.

- 12) **Ohta, T. :** Experimental Anticancer Studies. Part 6. Experiments on the Influence of Living A Group Hemolytic Streptococci and Several Other Species of Microorganisms on the Invasion Power of Ehrlich Carcinoma Cells to Mice. *Japan. J. Exp. Med.*, **27**, 107, 1957.

In the present work, living hemolytic streptococci and several other species of microorganisms were tested for their influence upon the invasiveness of Ehrlich carcinoma cells to mice.

The principal results obtained are summarized as follows :

1) Indications were that the activity of A group hemolytic streptococci to inhibit the invasiveness of the carcinoma cells to mice differs from strain to strain.

2) *Str. viridans*, *Str. faecalis*, *Str. liquefaciens*, *Staphylococcus albus*, Type II *Pneumococcus*, *E. coli* and *N. gonorrhoeae*. were all found to be entirely ineffective.