

# ABSTRACTS

1. STUDY ON THE ADJUVANT ACTION OF YEAST RIBONUCLEIC  
ACID ON THE BCG VACCINATION IN MICE

MASAHIRO YOSHIMURA, YASUE SHINAGAWA

AND TOSHIHIRO NANBU

*Department of Pharmacology, The Research Institute of  
Tuberculosis, Kanazawa University  
(Director : Prof. Ryo ITO)*

*Received for publication, May 30, 1966.*

Experiments were carried out to test the adjuvant action of yeast ribonucleic acid (RNA) on the BCG vaccination in dd mice.

Groups of mice, each consisting of 20—35 animals weighing 11—14 gm, were vaccinated by intraperitoneal injection of either BCG (about  $10^7$  cells) alone or with vaccine containing 0.001—10 mg RNA. Untreated control groups were included in every experiment. The mice were tested for immunity at various times up to one month after administration of vaccine, by intravenous injections of virulent human tubercle bacilli H<sub>37</sub>Rv (about  $10^7$  cells). The mice which died after the challenge inoculation were examined for tuberculous of the visceral organs.

The results of the experiments showed that the vaccines, to which RNA was added in doses of about 0.1 mg, were capable of producing a more marked prolongation of survival times in the mice than did that vaccine without the addition of RNA.

2. ANALYTICAL STUDIES ON TUBERCULIN REACTION

PART 2. THE TAXONOMIC KINSHIP OF VARIOUS MYCOBACTERIA  
AS STUDIED BY MEANS OF TUBERCULIN REACTION.

YUZO FUKUYAMA

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University  
(Director : Prof. Toshio SAITO)*

*Received for publication, June 15, 1966.*

For the purpose of clarifying the taxonomic kinship in eight strains of mycobacteria, comparative skin tests using o-Aminophenol Azo-Tuberculin (AT) prepared from each strain were carried out on guinea pigs sensitised with each of the strains.

The kinship of these bacilli was analysed by comparing the tuberculin reactions tested with several dilution series of AT's.

Tested in a high concentration of AT's, the sensitised animals were divided into two groups; the one reacting to slow growers' AT's (group S) and the other

to rapid growers' AT's (group R). The former was divided into subgroups TB and ATM and the latter into subgroups R1 and R2 in a lower concentration of AT,s. In further lower concentrations of AT's, each animal showed only a specific reaction to the homologous AT.

The classification of mycobacteria studied by means of comparative skin tests was considered to agree with biological or biochemical classifications.

### 3. EFFECT OF X-IRRADIATION ON DEVELOPMENT OF IMMEDIATE AND DELAYED SKIN HYPERSENSITIVITIES

SHINPEI TADA

*Department of Immunopathology, The Research  
Institute of Tuberculosis, Kanazawa University*

*(Director : Prof. Toshio SAITO)*

*Received for publication, Feb. 1, 1966.*

Experiments were carried out on five groups of guinea pigs for the purpose of clarifying the effect of whole body X-ray irradiation in nearly lethal doses on the development of immediate and delayed types of skin hypersensitivity reaction.

Group 1. Animals sensitized with hen egg albumin (HEA) in Freund's type adjuvant as controls.

Group 2. Animals X-rayed one day after sensitization with HEA in Freund's type adjuvant.

Groups 3,4 and 5. Animals sensitized with HEA in Freund's type adjuvant 1,3 and 7 days respectively after the irradiation.

Each group consisted of 2 subgroups, one sensitized with HEA in Freund's type complete adjuvant (HEA in FCA) and the other with HEA in Freund's type incomplete adjuvant (HEA in FIA). The results obtained were as follows:

1. Animals of Group 1 developed Jones-Mote type DHS (JM-DHS) to HEA on the 3rd or 4th day after the sensitization and IHS on the 9th or 10th day. Even after the development of IHS, the existence of tuberculin type DHS (T-DHS) was clearly demonstrated in animals sensitized with HEA in FCA, but not in those sensitized with HEA in FIA.

2. Animals of Groups 2 and 5 developed IHS almost at the same time as those of Group 1, whereas animals of Groups 3 and 4 developed IHS later than those of Group 1, and the shorter the interval between irradiation and sensitization, the longer was the latent period.

3. No perceptible difference was observed in the development of JM-DHS or T-DHS among the groups, that is, animals X-rayed either before or after antigen injection developed delayed skin hypersensitivity as early as the control animals

not X-rayed.

Thus, it was speculated that JM-DHS, T-DHS and IHS are probably mechanically different immune responses to sensitizing antigen, and that there is an obligatory sequential relationship among the three responses.

#### 4. THE EFFECT OF AN UNRELATED DELAYED TYPE SKIN REACTION ON DEVELOPMENT OF ANOTHER DELAYED TYPE SKIN REACTION

SHIZUKA SASAKI

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University  
(Director : Prof. Toshio SAITO)*

*Received for publication, April 11, 1966.*

The following seven groups of guinea pigs were employed in the experiments carried out for the purpose of studying the effect of a delayed skin reaction to the first antigen on the development of a delayed skin reaction to the second antigen injected intradermally together with the first antigen.

Group 1. Pretreated with hen egg albumin emulsified with Freund's complete adjuvant. (HEA in FCA).

Group 2. Pretreated with HEA emulsified with Freund's incomplete adjuvant (HEA in FIA).

Group 3. Pretreated with rabbit  $\gamma$ -globulin in FCA (RGG in FCA).

Group 4. Pretreated with RGG in FIA.

Group 5. Pretreated with tuberculoprotein in FIA (PF in FIA).

Group 6. Pretreated with FCA.

Group 7. Pretreated with wax D from human tubercle bacilli, H<sub>37</sub>Rv.

On day 0 or 21 after the pretreatment into the hind foot pads, all the groups were sensitized by intradermal route in the back as follows : Groups 1 and 2 were injected with RGG or with RGG added to HEA, Groups 3 and 4 with HEA or with HEA added to RGG, Groups 5 and 6 with HEA or with HEA added to OT, and Group 7 with HEA.

The results obtained are summarized as follows:

No differences in the development of delayed skin reaction to the second antigen in all the groups, whether injected alone or injected with the first antigen, were observed, but an apparent enhancement of the development of delayed skin reaction to the second antigen was observed in Groups 1, 3, 6 and 7 as compared with Groups 2, 4 and 5. The enhancement was attributable to the specific sensitization of the animals to wax D in the Mycobacterium contained in Freund's adjuvant.

5. ADJUVANT EFFECT OF ALUM AND BORDETELLA  
PERTUSSIS ON THE DEVELOPMENT OF  
DELAYED SKIN HYPERSENSITIVITY

MICHIKO KOSHIZAWA, SHIZUKA SASAKI,  
MICHIO TAKANISHI and TSUGIO SATOH

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University  
(Director : Prof. Toshio SAITO)*

*Received for publicaiton, April 25, 1966.*

It is a well known fact that after the administration of antigen with Freund's complete adjuvant, the development of delayed skin hypersensitivity precedes that of immediate skin hypersensitivity in guinea pigs. In the present study carried out for the purpose of clarifying the relation between delayed and immediate types of skin hypersensitivity, the effect of alum or Bordetella pertussis on the development of the two types of hypersensitivity was compared with that of Freund's complete type adjuvant, and it was clearly demonstrated that alum and Bordetella pertussis had little adjuvant effect on the development of delayed skin hypersensitivity. Thus, it was speculated that there is no obligatory sequential relationship among delayed and immediate types of skin hypersensitivity.

6. A HEMAGGLUTINATION PHENOMENON BETWEEN TANNED  
ERYTHROCYTES AND THE SERUM OF GUINEA PIGS

PART 6. THE EFFECT OF MANGANESE ON TANNED-  
ERYTHROCYTE AGGLUTINATION

FUMIHIKO MIKAMI

*Department of Pharmacology, The Research Institute  
of Tuberculosis, Kanazawa University  
(Director : Prof. Ryo ITO)*

*Received for publication, May 11, 1966.*

The effect of  $Mn^{2+}$  on tanned erythrocyte (TE-) agglutination of normal and tuberculous guinea pig sera was tested by mixing serial dilution of  $MnCl_2$  ( $5 \times 10^{-3} M - 5 \times 10^{-7} M$ ) with a dilution of serum. Saline solution buffered with a 0.1 M veronal-HCl mixture, pH 7.0, was used as a diluent.

The data obtained showed that  $Mn^{2+}$  has two types of action on TE-agglutination, i. e., inhibition and stimulation, the effect being very variable from serum to serum and, in the same serum, from dilution to dilution. In one serum  $Mn^{2+}$  exclusively exhibited a strong inhibition (minimum inhibitory concentration =  $10^{-6}$

M), yet in another serum it had a biphasic action, producing an inhibition at lower concentrations preceded by a marked stimulation at higher concentrations (more than  $10^{-4}$  M). The stimulation of TE-agglutination by  $Mn^{2+}$  was absent when a serum previously heated at  $56^{\circ}C$  for 30 minutes was used. It appeared that the variability of  $Mn^{2+}$  effect was more marked in the sera from tuberculous guinea pigs than from normal ones.

Some discussions were made concerning the mechanisms responsible for the variability of the  $Mn^{2+}$  effect.

## 7. ACQUIRED IMMUNOLOGICAL TOLERANCE OF SHEEP AND GOAT RED CELLS, WITH SPECIAL REFERENCE TO NATURAL ANTIBODIES

MICHIO TAKANISHI

*Department of Immunopathology, The Research Institute of  
Tuberculosis, Kanazawa University  
(Director : Prof. Toshio SAITO)*

*Received for publication, Feb. 17, 1966.*

An adult rabbit receiving sheep red cells and another receiving goat red cells produce 3 kinds of antibodies each. Two out of the 3 are antibodies responsible for both sheep and goat red cells,—one for Forssman antigen (F) and the other for another common antigen (B) to the red cells of both species. The third antibody is specific to the particular antigen of sheep or goat red cells (S or G).

The animals which had received "tolerance" injection of sheep or goat red cells in neonatal life were comparable with the untreated control animals in producing Forssman antibody, but inferior in producing anti-B and anti-S, or anti-B and anti-G, as the case might be.

Rabbits naturally develop anti-F in the course of maturing but no anti-B, anti-S or anti-G.

These data suggest that it is difficult to suppress natural antibody production by means of "tolerance" injection, but less difficult to suppress immune antibody production.

## 8. FUNDAMENTAL STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS

### PART 71. ANTITUBERCULOUS ACTION OF NITROFURAN DERIVATIVES

#### NO. 1. IN VITRO EXPERIMENTS

HAJIMU ASAJI

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University  
(Director : Prof. Masamichi KAKISHITA)*

*Received for publication, March 25, 1966.*

The in vitro antituberculous activity of various nitrofuran derivatives was studied, and the results obtained were as follows:

1) The growth inhibitory potency of eight derivatives to human type tubercle bacilli H<sub>37</sub>Rv was examined in various culture media. Q-ran, tested in Kirchner liquid media, Dubos liquid media and Kirchner agar media, showed the strongest antituberculous activity followed by 3-Miranon-M and Panfuran-S, however, all three derivatives showed considerably less antituberculous activity in Ogawa's 1% media and Sauton media.

2) In experiments testing the growth inhibitory activity of Q-ran, 3-Miranon-M and Panfuran-S to various acid-fast bacilli, it was demonstrated that a) all of the derivatives showed a most remarkable activity to human type tubercle bacilli among the various acid-fast bacilli tested and b) the antituberculous potency of these derivatives to SM-, PAS- or INH-resistant tubercle bacilli was equal to the susceptible ones.

3) In the successive cultivation of H<sub>37</sub>Rv in Dubos liquid media containing Panfuran-S or 3-Miranon-M, no change in the sensitivity of H<sub>37</sub>Rv to respective derivatives was demonstrated, while successive cultivation in media containing Q-ran showed a slight decrease in the sensitivity to Q-ran.

4) The effect of Q-ran, 3-Miranon-M or Panfuran-S on the in vitro development of H<sub>37</sub>Rv resistant to SM or INH was tested, and the three derivatives were all found to be effective for retarding the appearance of SM-resistant bacilli, of which Q-ran was most effective. However, all of them were ineffective against the appearance of INH-resistant bacilli.

5) As for the in vitro synergistic effects of the antituberculous action between Q-ran, 3-Miranon-M or Panfuran-S and SM or INH, all of the nitrofuran derivatives were, to some extent, synergistic with SM, whereas all of them but Panfuran-S were little synergistic with INH.

## 9. COMPARATIVE STUDY CONCERNING THE X-RAY EFFECT ON PRIMARY AND SECONDARY ANTIBODY RESPONSES

KISUKE ITO

*Department of Rapiology, School of Medicine,  
Kanazawa University  
(Director : Prof. Hiroshi HIRAMATSU)*

*Department of Immunopathology, The Research Institute of  
Tuberculosis, Kanazawa University  
(Director : Prof. Toshio SAITO)*

*Received for publicaiton, May 2, 1966.*

Rabbits naturally develop Forssman antibodies but no isophile antibodies to goat red cells. After the injection of the cells rise of Forssman antibody titer and production of isophile antibody is observable. Keeping these facts in mind, experiments were carried out to investigate the effect of X-rays on the primary and secondary responses to the Forssman and isophile antigens.

The results obtained were as follows:

1) Natural Forssman antibody titer began to fall and reached a minimum 3—4 days and 10—14 days respectively after being subjected to X-ray (200 to 600 r). The more the dose of X-rays the greater was the decrease of the antibody titer and the longer it took. Injection of goat red cells one day after X-ray reduced the degree and the period of the decrease and later raised the antibody production to a slight extent.

2) X-ray one day prior to the injection inhibited the primary and secondary responses, but the inhibition was less in the latter.

3) The inhibition of the primary response due to X-rays one day prior to antigen injection was reduced partially by reinjection of the antigen 7 days later and completed by the reinjection 14 to 21 days later.

4) Post-antigen X-rays have little effect on the primary and secondary responses except for a short prolongation of the latent, but occasionally enhancement of the primary response was observed while no enhancement of the secondary one.

5) The secondary response of animals which received pre-antigen or post-antigen X-ray at primary immunization was found to be almost equal to that of control animals not X-rayed at primary immunization.

From these data it was deduced that X-ray damage is largely limited to the first part of the antibody response. The recovery of the response from the X-ray damage was observed to begin several days after X-irradiation.

Accordingly, it is well recognized that the suppression of the primary response due to pre-antigen X-ray is rapidly recovered by means of the reinjection of the antigen after a proper period of time and that the immunological state responsible for the antigen stimulus by the secondary response is relatively resi-

stant to X-ray.

## 10. MASS EXAMINATION FOR THE ADULT DISEASE

### PART 3

KENSUKE MURASAWA

*Department of Surgery in Clinical Research, The Research Institute  
of Tuberculosis, Kanazawa University*

KOZABURO HONDA

*Kamioka Town Hospital, Gifu Prefecture*

KUNIO TOMITA

*Mitsui Mine Hospital in Kamioka Town*

*Received for publication, march 30, 1966.*

Mass examination was carried out for stomach disease and hypertension in Kamioka Town, Gifu Prefecture.

The reports for 1961 and 1962 were published already as part 1 and part 2. This paper, part 3, covers the years 1963 and 1964.

#### 1. Stomach disease

960 adults over 40 years of age and 1,086 adults over 40 years of age were examined for stomach disease in 1963 and 1964 respectively.

Among these subjects, 3 were found with suspected stomach cancer, 6 with a stomach ulcer, 5 with a suspected stomach ulcer, 1 with a stomach and duodenal ulcer, 13 with a duodenal ulcer, 7 with a suspected duodenal ulcer, 78 with chronic gastritis, and 126 with gastroptosis in 1963. In 1964, 1 with stomach cancer, 3 with suspected stomach cancer, 5 with a stomach ulcer, 7 with a suspected stomach ulcer, 1 with a stomach and duodenal ulcer, 15 with a duodenal ulcer, 16 with a suspected duodenal ulcer, 118 with chronic gastritis, and 158 with gastroptosis were found.

The one patient with stomach cancer was in poor condition. Upon operating or more exact examination, the 6 patients with suspected stomach cancer were diagnosed as having chronic gastritis or stomach ulcer. After operating, one case with a suspected palpable tumor in the abdomen was diagnosed as malignant Insuloma.

#### 2. Hypertension

1,177 adults over 30 years of age and 1,514 adults over 30 years of age were examined for hypertension in 1963 and 1964 respectively.

The percentage of those with systolic and diastolic hypertension increased with age. When the subjects were classified according to Master's limits of blood pressure, the percentage of those with systolic hypertension began to increase at age 55 in males and 50 in females. Diastolic hypertension began to increase at 55 in females. Prehypertension, i. e. latent hypertension appeared in the systolic pres-

sure of males at 40, while it appeared in the females at 50.

The subjects with hypertension and cardiac deformation revealed by chest roentgenography were males with systolic hypertension and females with diastolic hypertension.

11. MASS EXAMINATION FOR ADULT DISEASE AMONG  
THE STAFF AND EMPLOYEES OF HIGH SCHOOLS  
IN ISHIKAWA PREFECTURE PART 1

KENSUKE MURASAWA AND YOSHINORI MATSUMOTO  
*Department of Surgery in Clinical Research, The Research Institute of  
Tuberculosis, Kanazawa University*

YUZO FUKUYAMA  
*Department of Bacteriology and Immunology, Research Institute of  
Tuberculosis, Kanazawa University  
(Director : Prof. Masamichi KAKISHITA)*

*Received for publication, march 30, 1966.*

Mass examination carried out for stomach disease and hypertension among the staff and employees of high schools in Ishikwa Prefecture and the following results were obtained.

1. Stomach disease

546 adults over 40 years of age were examined for stomach disease.

Among these subjects, 3 were found with a stomach ulcer, 4 with a duodenal ulcer, 6 with a suspected duodenal ulcer, 2 with a stomach and duodenal ulcer. All of them were male. 39 were found with chronic gastritis and 62 with gastroptosis.

The cases of gastritis were more frequent in males and those of gastroptosis more frequent in females.

2. Hypertension

546 adults over 40 years of age were examined for hypertension.

The percentage of those with systolic and diastolic hypertension increased with age. When the subjects were classified according to Master's limits of blood pressure, prehypertension, i. e. latent hypertension appeared at age 50 and hypertension began to increase at age 55.

12. MASS EXAMINATION FOR ADULT DISEASE AMONG  
THE STAFF AND EMPLOYEES OF HIGH SCHOOLS  
IN ISHIKAWA PREFECTURE PART 2

KENSUKE MURASAWA AND YOSHINORI MATSUMOTO

*Department of Surgery in Clinical Research, The Research Institute of  
Tuberculosis, Kanazawa University*

YUZO FUKUYAMA

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University*

*(Director : Prof. Masamichi KAKISHITA)*

*Received for publication, march 30, 1966.*

Mass examinations for stomach disease and hypertension were continuously carried out from 1963 through 1965. Part 1 of this study represents the findings for 1963. Part 2 covers 1964 and 1965.

1. Stomach Disease

389 adults over 40 years of age and 323 adults over 40 years of age were examined for stomach disease in 1964 and 1965 respectively.

Among these subjects, 3 were found with a suspected stomach ulcer, 13 with a duodenal ulcer, 10 with a suspected duodenal ulcer, 1 with a suspected polype of the stomach, 44 with gastritis, and 14 with gastritis in 1964. In 1965, 3 with suspected stomach cancer, 1 with a stomach ulcer, 1 with a suspected stomach ulcer, 2 with a duodenal ulcer, 5 with a suspected duodenal ulcer, 1 with a stomach and duodenal ulcer, 35 with gastritis and 20 with gastroptosis were found.

The 3 patients with suspected stomach cancer were diagnosed as chronic gastritis after use of the gastroscope or more exact examination. Thus, there were no patients with stomach cancer. Cases of ulcers and gastritis were more frequent in males while those of gastroptosis more frequent in females as was the case in 1963.

2. Hypertension

389 adults over 40 years of age and 323 adults over 40 years of age were examined for hypertension in 1964 and 1965 respectively.

The percentage of those with systolic and diastolic hypertension increased with age. When the subjects were classified according to Master's limits of blood pressure, prehypertension, i. e. latent hypertension appeared at age 50 and hypertension began to increase at 55 as in 1963.

13. MASS EXAMINATION FOR ADULT DISEASE IN MEMBERS  
OF THE STAFF OF THE PREFECTURAL OFFICE IN  
TOYAMA PREFECTURE PART 1

KENSUKE MURASAWA, YOSINORI MATSUMOTO AND  
TOKIO KAMIHARA

*Department of Clinical Research, The Research Institute of  
Tuberculosis, Kanazawa University*

YUZO FUKUYAMA

*Department of Bacteriology and Immunology, The Research  
Institute of Tuberculosis, Kanazawa University  
(Director : Prof. Masamichi KAKISHITA)*

MOICHI SUZUKI AND SHIGERU MASAOKA

*Toyama Juzen Hospital in Toyama City  
(Director : Moichi SUZUKI, M. D.)*

HAJIME YANO

*Chief of Personal Division in Toyama  
Prefectural Office in Toyama  
(Toyama Prefectural Governor: Minoru YOSHIDA)*

*Received for publication, march 30, 1966.*

Mass examination was carried out for stomach diseases and hypertension in Toyama Prefecture.

1. Stomach Disease

519 adults over 40 years of age were examined for stomach disease. Among the subjects, 2 were found with suspected stomach cancer, 8 with a stomach ulcer, 10 with a suspected stomach ulcer, 4 with a duodenal ulcer, 9 with a suspected duodenal ulcer, 79 with gastritis, and 21 with gastroptosis.

Upon operating or more exact examination, the 2 patients with suspected stomach cancer were later diagnosed as having chronic gastritis. Thus there were no patients with stomach cancer.

2. Hypertension

663 adults over 30 years of age were examined for hypertension. The percentage of those with systolic and diastolic hypertension increased with age.

When the subjects were classified according to Master's limits of blood pressure, the percentage of those with systolic and diastolic prehypertension began to increase at age 55.

Most of the subjects with hypertension and cardiac deformation revealed by the chest roentgenography were patients with hypertension over 110 mmHg of diastolic pressure.

## 14. A CASE OF BENIGN FIBROMA OF THE CHEST WALL

KENSUKE MURASAWA, \*SEIICHIRO HAYASHI

AND \*TATSUO NAGAJI

*Department of Clinical Research, The Research Institute of  
Tuberculosis, Kanazawa University*

AKIO MUKAWA

*Department of Pathology, School of Medicine,  
Kanazawa University**(Director : Prof. Tachio ISHIKAWA)*

SHIGEMI MASAOKA

*Toyama Juzen Hospital in Toyama City  
(Director : Moichi SUZUKI, M. D.)**Received for publication, march 30, 1966.*

A case of benign fibroma of the chest wall was reported on 31-year old male.

The chest X-ray showed two isolated round tumorous shadows in the left upper and middle fields which had not produced any complaints until they were discovered by mass examination on December 8, 1964.

After admission to Juzen Hospital on December 29, 1964, he was treated with SM, PAS and INH as pulmonary tuberculoma, but the antituberculous therapy had no effect upon the X-ray shadows. Tubercule bacilli and malignant tumor cells were not found in the sputum. Bronchography revealed no pathological changes.

Left thoracotomy was performed on March 4, 1965 with a tentative diagnosis of chest wall tumor and it revealed two isolated, well circumscribed round soft tumors in the left posterior chest wall. Resection of the tumors was succeeded and they were diagnosed histopathologically as a benign fibroma of the chest wall. There was no invasion of the tumor in the surrounding tissue nor metastasis to the regional lymphnodes.

Fibroma in the soft tissue of the chest wall is a rare occurrence and the clinical signs except for positive incidental findings on chest X-ray. For effective treatment surgical excision has been indicated because recurrent tendency or malignant transformation of some of the fibroma is noted.

---

\*At present: Department of Surgery, School of Medicine, Kanazawa University