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## Studies on the Antitubercular Compounds (XI)

### On the PAS Salt of INAH

Shichiro KAKIMOTO and Ken-ichi YAMAMOTO

Recently it has been reported by H. Brodhage that the p-aminosalicylate (PAS) salt of isonicotinic acid hydrazide (INAH) is unexpectedly highly active in *in vitro* tests against *Mycobacterium tuberculosis* H37Rv and certain drug-resistant strains.

In the present study, attempts were made to obtain salts of some substances related to PAS and INAH under the same condition as that for the preparation of PAIN. Table 1 shows the results which lead one to presume that the new salt may be dibasic rather than monobasic.

The results of antituberculous activity *in vitro* are summarized in Table 2. Brodhage reported that PAS salt of INAH possesses a superior tuberculostatic effect even against strains resistant to both PAS and INAH, but the data obtained in the present study did not agree with his data. Moreover, the drug was found to be completely inhibitory against strain H37Rv at 17 per ml. being more active than INAH. SAIN was as active as the latter.

It is interesting that PAIN showed a tuberculostatic activity greater than a mere mixture of equivalent amounts of PAS and INAH. This would indicate that PAIN is a new chemical compound.

## On the Applicability of Verhulst's Logistic Curve to the Growth Curve of *Mycobacterium Tuberculosis*

Tomowo OGITA

Verhulst's logistic curve was found to be applicable to the growth curve of BCG cultivated in Kirchner's liquid media, when it was examined by the turbidimetric method or by the micro-Kjeldahl nitrogen determination.

## Quantitative Studies on the Complement Fixation Reaction by the Unit of 50 Per Cent Hemolysis with Special Regard to the Titrating Method of Hemolysin and Comparison of Various Antigens to Tuberculous Sera

Takashi IKEHATA

In succession to previous papers, this paper deals with the study made on the methods of complement fixation test in tuberculosis by the unit of 50 per cent hemolysis.

A) A new procedure has been devised to determine objectively the optimum concentration of hemolysin to be used in the test. Namely, the amount of complement necessary for giving 50 per cent hemolysis was obtained for each red cell suspension sensitized with varying dilutions of hemolysin. Then the sum of the undiluted quantity of the complement used and that of the hemolysin concerned was calculated respectively in each case. The dilution of hemolysin used in the case where the calculated value of the sum is minimum, was taken to be the optimum concentration.

B) In titrating sera of rabbits experimentally infected with tuberculosis, use as antigen of a methanol extract from tubercle bacilli defatted with acetone (Boquet and Nègre's antigen) and an old tuberculin

etc. led to the following results.

- 1) Methanol-extracted and ethanol-extracted antigens were of the same behavior towards the same antituberculous sera.
- 2) Old tuberculin revealed two field dimensions of reaction.
- 3) The methanol insoluble fraction of old tuberculin proved to possess the same reactivity as that of old tuberculin itself.
- 4) When the methanol extract of tubercle bacilli was heated after the same quantity of 1 % KOH was added, its antigenicity disappeared.
- 5) On the contrary, even after treatment by the same procedure, old tuberculin maintained well its antigenicity, without showing any antigenic alteration.

## Influence of Egg-Yolk on the Virulence of Mycobacterium Tuberculosis

### II. Examination on the method and the site of egg-yolk administration

Ken-ichi YAMAMOTO and Mikio ITO

It was shown in a preceding paper that egg-yolk intensifies tuberculin allergy induced in animals and enhances tuberculous lesions caused by inoculation of virulent tubercle bacilli. However, the mechanism of the enhancing action of egg-yolk on the virulence of the tubercle bacillus has not been clarified. The present study was undertaken to find out some of the enhancing factors on the part of the host.

Three groups of guinea pigs were inoculated subcutaneously with one single injection of 0.01 mg of virulent bacilli, strain Nakano, suspended in 50 % egg-yolk solution for one group and in distilled water for the remainder groups. One of the latter groups received at the same time and subcutaneously 0.2 ml of egg-yolk separately from the site of bacillary injection. The animals of this group received two injections of egg-yolk of the same dose, once a week.

In another experiment, guinea pigs were infected through the intraperitoneal route with 0.001 mg of virulent bacilli suspended in 50 % egg-yolk solution. An equal number of control animals received the same dose of bacilli suspended in distilled water.

After inoculation, all the animals were observed as described in the preceding paper.

The experiment led to the following results:

1. Egg-yolk, exhibits an effect similar to that observed in the preceding experiment, even when administered intraperitoneally.
2. Egg-yolk enhanced tuberculous lesions in the visceral organs and the lymph nodes to the some extent, if it is given mixed with virulent bacilli or separately from the latter.

## Influence of Egg-Yolk on the Virulence of Mycobacterium Tuberculosis

### III. Influence of egg-yolk on the protective effect of BCG against infection with virulent tubercle bacilli

Ken-ichi YAMAMOTO and Tetsuji HASHIMOTO

Guinea pigs were divided into three groups of equal number, one receiving 0.001 mg of BCG suspended in distilled water, another the same dose of BCG suspended in 50 per cent egg-yolk solution and the third 0.1 mg suspended in distilled water. After vaccination, local lesions at the site of vaccination and tuberculin skin reactions were examined and recorded weekly. Five weeks after vaccination all the animals, together with the control animals, were challenged subcutaneously by the injection of 0.001 mg of virulent, human-type tubercle bacilli, strain Nakano. They were sacrificed 8 weeks after challenge and a

comparative observation was made grossly as well as histologically of tuberculous lesions in the visceral organs and the lymph nodes. Quantitative cultures were made for comparison of the spleens of all the animals. Thus, the immunity induced in the animals by vaccination with BCG was measured by the two criteria. a) Diminution of tuberculosis lesions in the visceral organs and the lymph nodes noticed after challenge in the vaccinated animals. b) Difference in the number of living bacilli recovered from the spleens of the vaccinated and control animals after infection with virulent bacilli.

The experiment led to the following results:

1. Egg-yolk does not enhance local lesions at the site of vaccination with BCG.
2. Egg-yolk intensifies tuberculin allergy induced in animals by vaccination with BCG.
3. The immunity produced in animals by vaccination with BCG suspended in egg-yolk solution is of the same degree as to be produced by a hundredfold amount of BCG suspended in distilled water.
4. The present results suggest that the immunity induced by BCG vaccination is directly related to the extent of multiplication of the bacilli in the tissues of the vaccinated animal.

**Influence of BCG and Killed Tubercle Bacilli  
on the Development of Tuberculous Infection in Guinea Pigs  
with Special Reference to Concomitant Inoculation  
of BCG or of Killed Bacilli and Living Virulent Tubercle Bacilli**

Jun ARIMA, Ken-ichi YAMAMOTO and Kazuo MORIKAWA

The immunizing potency of BCG bacilli and heat killed tubercle bacilli against tuberculous infection was studied for comparison by mixing them with the living virulent tubercle bacilli destined for subcutaneous inoculation in guinea pigs.

The results obtained were as follows:

- 1) A suppressive effect of BCG on tuberculous infection was noticed, when the amount of BCG mixed was approximately hundred times as large as that of the virulent bacilli incorporated.
- 2) The suppressive effect of BCG was also recognized even when inoculated simultaneously with but separately from the virulent bacilli.
- 3) In case of concomitant inoculation the multiplication of the virulent bacilli in the local tissue was suppressed for several days, while in the visceral organs, the virulent bacilli which were found to be disseminated about ten days later than BCG bacilli, decreased in number first rapidly, then gradually. The present author is of the opinion that this effect of BCG produced following its enormous inoculation is an expression of immunity.
- 4) Contrary to what happened with BCG, heat killed tubercle bacilli enhanced tuberculous infection, when mixed with living virulent bacilli and inoculated subcutaneously into animals. The amount of killed bacilli necessary for producing this phenomenon was approximately ten thousand times as large as that of the living virulent bacilli incorporated.
- 5) On the other hand, killed bacilli manifested little enhancing effect in the following cases: in the case when animals were inoculated simultaneously but separately with killed bacilli and living virulent bacilli, and in the cases where killed bacilli were inoculated at several day intervals within comparatively short period of time before or after challenge infection.
- 6) A certain correlation was considered to exist between the infection-enhancing effect of killed bacilli and the virulence of the strain used for their preparation.
- 7) In the local tissue as well as in the visceral organs, the living virulent bacilli inoculated increased in number more rapidly in the concomitant inoculation group than in the control.

Histologically tuberculous changes in the organs were found to be severe in the former than in the latter. However, qualitatively, there was found no difference between both groups.

- 8) Finally, heat killed bacilli exhibited a certain degree of immunizing potency in the case where they

were inoculated into animals with their adequate amounts and during an adequate period of time before challenge infection.

From the data thus obtained, the heat killed tubercle bacillus was found to exhibit two different actions, infection-enhancing and immunizing, according to the methods of inoculation.

### Influence of Antituberculous Chemotherapeutica on the Various Enzymic Activities and Viability of BCG

Ken-ichi YAMAMOTO

Comparative observation was made of the effect of streptomycin (SM), isonicotinic acid hydrazide (INAH) and para-aminosalicylic acid (PAS) on the oxygen consumption, catalase activity, lactico-dehydrogenase activity, tetrazolium-salt reducing activity and viability of BCG, both in its growing and resting condition in order to explain the mechanism underlying the action of these drugs.

The experiment led to the following results.

1) The bactericidal action toward BCG growing on Sauton media is far more remarkable for SM than for INAH. However, the tetrazolium-salt reducing activity and catalase activity are both reduced significantly by both drugs, respectively. The oxygen consumption and lactico-dehydrogenase activity is inhibited by INAH. Having little bactericidal effect, PAS does not almost inhibit the various enzymic activities.

2) The bactericidal action of SM and its inhibitory action toward the various enzymic activities of BCG vary significantly with its amounts to be used. In the case of INAH, such a variation is not observed.

3) In Kirchner submerged medium, the effect of the drugs on the enzymic activities of BCG culture is demonstrated just in the case of Sauton media, but their comparable bactericidal actions are much reduced,

4) The bactericidal action of SM increases for vigorously growing cultures of BCG.

5) The viability of BCG does not seem to be closely related to the inhibition of its enzymic activities by the drugs.

6) Considering that the drug effects above mentioned are not secured in bacilli resistant to that the corresponding drugs, the present author is of the opinion that the drug actions observed are specific to the bacilli.

### Variation in Various Immunological Reactions Following BCG Vaccination as Realized through Different Routes of Inoculation

Ken-ichi YAMAMOTO and Akihiko KUZE

The experiments led to the following conclusions:

1) In case of primary vaccination, the precipitin titer and the hemagglutinin titer were the highest in the group of animals vaccinated intravenously, followed by the subcutaneous group. They were the lowest in the intracutaneous group. This tendency was similarly observed following revaccination and challenge with virulent tubercle bacilli.

No appreciable difference in the degree of cutaneous tuberculin reaction was noticed among these three groups of animals except for its somewhat late appearance in the intravenous group.

2) In case of revaccination, tuberculin skin allergy, after having showed once a decrease of a short duration, increased in intensity more rapidly than in case of primary infection, and the circulating antibody level became remarkably high, but its duration was relatively short.

3) After challenge, no appreciable difference was found in tuberculin allergy between the vaccinated groups and the control group. However, in the latter, the circulating antibody level was found to be remarkably low.

4) Acquired resistance was high in the order of: intravenous group, subcutaneous group and intracutaneous group.

5) No parallelism was noticed among intensity of tuberculous lesions, precipitin titer, hemagglutinin titer and degree of tuberculin skin allergy.

## Electrophoretic Studies on Tuberculous Sera

by the Use of Filter Paper (I)

Tatsuo NUMATA, Tomowo OGITA, Takao SHIDA and Masuo ITAKURA

1) In the electrophoretic pattern of rabbit sera, the percentage of the albumin fraction estimated by paper-electrophoresis is generally found to be lower than that estimated by Tiselius' apparatus. On the contrary, the percentage of the  $\gamma$ -globulin estimated by paper-electrophoresis is generally found to be higher than that estimated by Tiselius' apparatus.

2) On the whole, antibody titers estimated by precipitin reaction run parallel with the quantity of the  $\gamma$ -globulin estimated by the electrophoresis using either filter paper or Tiselius' apparatus, though the individual deviations are not so small.

## Tuberculosis and Urine Quotient

2. Urine quotients as new criteria for measuring the general adaptation faculty of human bodies under various conditions and its clinical application in the experimental therapy of tuberculous patients, with special reference to the effect of INAH-Methionine joint therapy.

Osamu NISHIKAZE, Yoshijiro KITAMURA and Hide TAKEUCHI

A clinical and metabolic study on tuberculous patients under treatment with INAH (Control group) and those receiving INAH-Methionine treatment was conducted in twenty-nine patients. Both INAH and Methionine were orally administered respectively at the rate of 4 mg per kg body weight and 3 g per 50 kg body weight per diem divided into two doses.

1) Clinical:

No favorable effect of INAH-Methionine joint therapy was obtained as compared to the control.

2) Metabolic:

a)  $O/K_4$  (a new criterion for measuring the general adaptation faculty of human bodies at given moments): in the control group,  $O/K_4$  value, showed a remarkable rise in the 2nd-3rd week, dropped during the intermediate weeks and rose moderately in the 9th-10th week, while in the INAH-Methionine group the initial rise was not noticed. Statistically both cases showed a high value as compared to the normal value:  $30.0 \pm 55.77$ .

b)  $Ka/K_4$  (a new criterion for measuring the intensity of the adrenal function): the resulting curve was almost identical to the above, both in the INAH and INAH-Methionine groups. Statistically both cases showed a high value as compared to the normal value:  $0.53 \pm 0.144$ .

c)  $Ku/K_4$  (a new criterion for measuring the intensity of liver damage): the resulting curve was identical to the above, but, as compared with the normal value of  $0.42 \pm 0.145$ , a statistically significant difference was noticed for the average of figures obtained for the treatment with INAH alone in

the 3rd and in the 10th week after the beginning of treatment, and for the joint treatment of INAH-Methionine in the 10th week after treatment.

It was concluded that INAH-Methionine joint therapy, results in the attenuation of the toxicity of INAH in the initial period of administration, but fails to produce any favorable result in the latter period.

### Pathological Study on Delayed Shock

#### 1. Anatomic-pathologic examination on delayed shock experimentally produced in rabbits.

Hiroshi TOKITA

Rabbits previously immunized with BCG or human tubercle bacilli were challenged with a large quantity of BCG or injected intravenously with old tuberculin. Six to forty-eight hour after challenge or injection the animals died showing severe shock-like symptoms.

Anatomic-pathologic examination of these rabbits led to the following results:

1. Remarkable swelling and congestion of the lungs and conspicuous enlargement and hypermeia of the spleen were observed grossly.
2. Microscopical observation of the lungs showed marked exudative changes: intense hyperplasia of large mononuclear cells, and exudation of polymorphonuclears in the alveoli, severe inflammatory edema and cell infiltration in the alveolar wall and characteristic leukocyte stasis in the small blood vessels. The bronchial lumen were narrowed due to swelling and desquamation of the epithelial layer and severe leukocyte infiltration.
3. The spleen showed enormous dilation in the sinus in which marked hyperplasia of large mononuclears and severe exudation of polymorphonuclears were observed. The lymph follicle of the spleen showed conspicuous karyorrhexia of the lymphocytes.
4. The liver showed dilatation in the sinusoid, in which a large quantity of polymorphonuclears and large mononuclear cells were observed.
5. In these rabbits no qualitative difference was detected between the change following BCG reinfection and those following tuberculin injection.