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Study on the Hemagglutination Reaction with Special Reference
to the Influence of Heating on the Antigens

Katsuo ONO and Yoshio TAKAHASHI

Proteinic fractions were isolated from Sauton culture filtrates of human tubercle bacilli (H 37 Rv),
by precipitation with trichloroacetic acid. Polysaccharine fractions were isolated from the deproteinized
filtrates by precipitation with methanol at concentrations of 30 to 40 and 80 to 90 percent. The
hemagglutination reaction was comparatively carried out with these fractions, according to Middlebrook­
Dubos' method and Boydon's. At the same time, influence of heating on the fractions was studied.
The results were as follows:
1. According to M.-D.'s method, both polysaccharine and proteinic fractions gave positive reactions,
while, according to B.'s method, only proteinic fractions did so.
2. Of the polysaccharine fractions, 80 to 90 percent methanol fractions gave positive reactions, while
below 40 percent methanol fractions did not. In the cross-inhibition test, these latter fractions showed
no inhibiting power to the reactions to be given by the former fractions.
3. Dialysis (3 days, running water) and mild hydrolysis with M/10 HCl exerted no appreciable
influence on the hemosensitizing power of the proteinic fractions.
4. Heating at 120°C during 2 hours decreased remarkably, especially in an alkaline medium, the
hemosensitizing power of the fractions, while their precipitin and inhibiting titres remained unchanged.

Study on the Blood Level of Isoniazid in Case
of Combined Chemotherapy

Tadazumi ONODERA, Tadashi KUWAJIMA,
Hajime FUKAE and Eiichi SAKAI

In order to get information of the mechanism of the effect of the current, various procedures of
combined chemotherapy for tuberculosis, the blood level of INH was measured with sera obtained
respectively from patients treated with INH alone, those treated with INH plus PAS, those treated with
INH plus pyrazinamide (PZA) and those treated with INH plus sulfisoxazole. The measurement was
done according to Ogawa's diffusion method (biological assay).
According to the method, no evidence was obtained that would indicate any elevation of the blood
level of INH in any of the combined treatments above mentioned. In the results obtained, the
individual differences were so great that it was impossible to draw any significant conclusion.
As for the development of INH-resistance, bacilli of high resistance were found in patients in whom
the blood level of INH was relatively high. Moreover, no correlation was found between development
of INH-resistance and doses of INH administered.
Study on the Mechanism of the Effect of Combined Chemotherapy of Sulfonamides and Isoniazid in Tuberculosis Patients

Makoto Shoji

Tuberculosis patients were orally administered the following four kinds of sulfonamides respectively, in combination with isoniazid (INA) and in equal molecular weight: sulfisoxazole, sulfisomidine, sulfathiazole and sulfamethoxypyridazine. Blood level of INA was measured according to the bromcyan method at regular intervals of time after the administration of the drugs. At the same time, the antibacillary activity of the sera was measured bacteriologically, using human type tubercle bacilli. The experiments led to the following results:

1. All the four sulfonamides tested raised almost equally the blood level of INA and prolonged the duration of its action.
2. As the antibacillary activity of these sulfonamides was found to be very low (30-50 μg/ml), the effectiveness of their combination to INA would be mainly due to the fact that the in vivo acetylation of INA is retarded in their presence.

Influence of Carbonic Acid Gas on the Action of Antituberculous Agents

Yoshitane Nagayama

Using Dubos' tween-albumin medium, human type tubercle bacilli, strain H 37 Rv, were cultured partially in the ordinary air and partially in carbonic acid gas. In both cases, the bacilli were periodically examined on the following points: growth, stainability, morphological features, sensitivity to streptomycin and isoniazid, mode of development of resistant bacilli, and relationship between electron-microscopically non-transparent granules (the so-called A body) and cytochemically stainable various granules. The experiment led to the following results:

1. The growth of the bacillus was suppressed by carbonic acid gas, its suppressing action being rather of bactericidal nature.
2. The action of SM and INH was found to be bactericidal to a certain extent.
3. The bacillus was found to be insensitive to SM and INH, when its growth was hindered in carbonic acid gas.
4. Resistant bacilli appeared in the ordinary air from the second week after cultivation, while no resistant bacillus developed in carbonic acid gas.
5. Decrease in acid-fastness was noticed following addition of INH, irrespective of the cultural condition of the bacillus. This phenomenon was likewise observed to some extent for SM and carbonic acid gas.
6. Electron-microscopically, evidence was obtained for increase in transparency of the bacillary bodies, gradual disappearance of the A bodies and autolysis of the bacilli, in the case of the bacilli subjected to the action of SM, INH and carbonic acid gas. Unusual swelling and extension of the bacillary bodies were also observed for the bacilli under the action of INH and SM in carbonic acid gas.
7. No correlation was found between the so-called A bodies, toluidin blue stainable granules and
janus-green B stainable ones. This indicates that the A bodies are neither of metaphosphatic nor of mitochondrial nature.

Comparison of the Precipitin Reaction with the Complement Fixation Reaction in Tuberculosis, with Special Reference to the Relationship between Their Antibody Titers and the Protective Action in Animals

Takashi IKEHATA, Tatsuwo NUMATA, Shirō ISHIYAMA and Goro HIRANO

Rabbits immunized with BCG were bled at regular intervals for the titration of antibody both by the precipitin reaction and the complement fixation reaction. Thus, the relationship between both antibody titers and the resistance of the animals against tuberculous infection was observed. The results obtained were as follows:

1) The degree of resistance of the animals against tuberculous infection runs parallel with the values of antibody titers estimated by the precipitin reaction. In this respect, it may be said that the precipitin reaction can be used as a criterion for measuring the degrees of immunity.

2) While antibody titers estimated by the precipitin reaction and those estimated by the complement fixation reaction have a definite correlation when estimated at the same point, the resulting curves of both reactions do not run parallel through the entire course of immunization.

3) The complement fixation reaction turns positive in the earlier stage of immunization and disappears rapidly after a short stationary period, whereas the precipitin reaction turns positive in the stage much later than the former and its antibody titers increase gradually up to the 2nd month after immunization. Accordingly, curves of the development of antibody titers estimated by these two serum reaction cross one another about one month after immunization.

4) Therefore, the relation between antibody titers estimated by the precipitin reaction and those estimated by the complement fixation can only be compared under definite experimental conditions, because it varies with the experimental methods and with the time of observation.

Studies on Tuberculin Allergy in Mice

Tatsuwo NUMATA and Satoshi FUJIMOTO

1) Tuberculous mice which have hitherto been considered as naturally anergic to tuberculin showed a positive tuberculin reaction, when ca. 0.06 ml of 25 fold dilution of old tuberculin was injected into their footpad.

2) About half the mice turned allergic to tuberculin 4 weeks after infection with tubercle bacilli, but the majority of them did so about 7 weeks after infection. This footpad reaction showed a tendency to weaken from the 10th week on.

3) When electrophoretically examined, sera from tuberculous mice showed a relative decrease in the β-globulin fraction in comparison with those from normal mice.

4) Sera from tuberculous mice gave a positive precipitin reaction towards water extract of tubercle bacilli.
Studies on Tuberculin Neutralizing Substance Present in the Organs of Rabbits Immunized with BCG

Shirō ISHIYAMA

This paper dealt with the existence of tuberculin neutralizing substance in the organs of guinea pigs immunized with BCG and rendered strongly tuberculin-positive.

For this purpose, lung-, liver- and spleen-extracts prepared from guinea pigs immunized with BCG were respectively added to old tuberculin in order to absorb the active principle involved in tuberculin and the mixtures were centrifuged. Then, the supernatants were injected, according to the arrangement of Latin Squares, into the skin of guinea pigs infected with tubercle bacilli. The resulting skin reactions were much less intense than those observed in the case of intracutaneous injections of tuberculin without any treatment. Thus, the organ extracts prepared from guinea pigs immunized with BCG proved to have an activity to neutralize tuberculin.

However, this activity was not demonstrated by the complement fixation test.

A study on the usage of blood transfusion, and transfusion solutions in surgical treatment of tuberculosis in present day Japan

Osamu NISHIKAZE, Tomio GOCCHO, Yoshigiro KITAMURA, Syohei SEKIGUCHI, Hide TAKEUCHI, Takashi OGURI and Yoshiyasu HARADA

Questionnaires were sent to hospitals and sanatoriums throughout Japan, at which lung operation methods are used as therapeutic measures. 150 replies were received and studies were made in order to investigate the present condition in Japan with special regards to blood transfusion and transfusion solutions. Two approaches to the problem; type of operation and geographical difference were used.

1) Results as seen through type of operation

A) Preoperatively, in half of the 150 cases, neither blood transfusions nor transfusion solutions were administered. As regards blood transfusion, in most cases, 200~400 cc per day were used on the day of the operation or one day prior to the operation. On the other hand as regards the usage of transfusion solution, in the majority of cases, 500~1000 cc per day are administered on the day of the operation. Ringer's solution and 5% glucose or an even mixture of the two are used in most cases.

B) During operation, in all types of operation both blood transfusion and transfusion solution are used. However, in the majority of cases, blood transfusion alone is used. As regards blood transfusion, in the lung resection group, 20~50% over the amount of blood lost is administered. In the thoracoplasty an amount equalling the blood lost is administered using stored blood in the most cases. On the other hand, in regards to the transfusion solution, 5% glucose or physiological saline solution is administered singly, while in the mixed solution, in the majority of cases, 5% glucose plus physiological saline solution or Ringer's solution is administered at rate of 500~1000 cc.

C) Postoperatively, both blood transfusion and transfusion solution are used in the resection group, whereas in the thoracoplasty there seems to be a tendency to use transfusion alone. In regards to blood transfusion, an equal amount to the pumped-out withdrawn is transfused on the day of the operation in the resection group, whereas, in the thoracoplasty, there is a tendency to use 200~400 cc per day on the
day of the operation and the following day. On the other hand with regards to transfusion solution, in the majority of cases, either 5% glucose or Ringer's solution are used singly and/or a mixture of the two are administered for 1~3 days.

2) Geographical (locality) differences

A) Preoperatively, in the Hokkaido and Kanto areas, in both resection and thoracoplasty cases, it was noted that both blood transfusion and transfusion solution were used in the majority of cases, whereas, in the Kinki area and further south, transfusion solution alone is used in the majority of cases. No geographical differences were seen in the number of days or the amount of blood transfusion or transfusion solution used. Further, it was noted that in the Kinki area the use of Ringer's solution is predominant.

B) During operations, in both resection and thoracoplasty cases, it was noted that in the Kinki area blood transfusion alone is used in the majority of cases. In regards to the quantity of blood transfused, there is a tendency to limit the quantity in Hokkaido. On the other hand, in regards to the quantity of transfusion solution, the usage in the presence of resection is lower in areas north of Kanto as compared with that of south of Kanto. As regards the type of transfusion solution the usage of 5% glucose in Kanto is significantly outstanding.

C) Postoperatively, it is noted that in the Kinki area blood transfusion only is used in resection cases, while in thoracoplasty cases transfusion solution alone is used. It is noteworthy that the type of transfusion is decided by the type of operation. As regards the number of days that blood transfusions are administered, Hokkaido shows alightly higher figures as compared against other areas. Likewise, in the number of days that transfusion solutions are administered, Hokkaido alone shows slightly higher figures as compared with other areas. Concerning the type of transfusion solution, 5% glucose is used in Hokkaido while in Tohoku, Kanto and Chugoku 5% glucose plus Ringer's solution is used mainly. And in the cases, the usage of Ringer's solution in the Kinki area is highly significant.

Influence of Adrenocortical Hormone on the Immunologic Reaction in Rabbits Inoculated with BCG

Tetsuji HASHIMOTO

Cortisone is reported to inhibit various kinds of allergic reactions, such as the Arthus phenomenon, anaphylaxis or atopic reactions, which are all classified in the hypersensitive reactions of immediate type. However, influence of cortisone on the delayed type reaction was still unkown. This has led the author to make the following experiments.

Predonin, Prednisolone-Shionogi Co., was daily injected into rabbits during the course of the experiment. On the 7th day after the beginning of the injection the animal were inoculated with BCG. At ten day intervals tuberculin skin tests were recorded and the sera of the animals were studied electrophoretically.

The results obtained were as follows: 1. Predonin markedly inhibited the development of tuberculin sensitivity and the severity of tuberculin reaction. 2. The serum precipitin titer to water extract of tubercle bacilli was in general low in the animals treated with Predonin. 3. The total protein concentration of the sera was low in the treated animals. 4. In control animals the serum γ-globulin fraction increased gradually, while in the treated animals the increase of the fraction was not remarkable. These data would indicate that Predonin inhibits the production of antibody to tuberculin.
Serological Study on Tuberculosis

V. Role of the Serum Protein Fractions in the Serological Reactions

Akio Hagiwara

In order to verify the relation of the serum protein fractions to precipitin and to the skin sensitizing antibody, the sera of rabbits sensitized to crystalline egg-albumin or inoculated with BCG were studied.

First, two globulin fractions, γ-globulin according to Cohn's method, and another globulin component were fractionated from the sera.

The precipitins to egg-albumin or old tuberculin were found only in the γ-globulin fraction.

Second, the fractions were injected intraperitoneally into normal guinea pigs or normal rabbits. Sixteen hours after injection, an 1 per cent egg-albumin solution or 40-fold old tuberculin was injected intracutaneously. The reactions thus provoked were measured repeatedly at regular intervals. A marked positive reaction occurred only in the animals injected with the γ-globulin fraction. In case of old tuberculin the reaction was of delayed type in rabbits, but it was not so in guinea pigs.

To certify these data zone electrophoretic experiments were performed. Sera obtained from rabbits sensitized with crystalline egg-albumin solution were analyzed by starch electrophoresis. The precipitating antibody to egg-albumin were found in the slow moving γ-globulin fraction, while the skin-sensitizing antibody in the fast moving γ-globulin fraction.