Exalting Influence of Egg-Yolk on the Virulence of Mycobacterium Tuberculosis.

Kenichi YAMAMOTO and KAZUO MORIKAWA

Virulent, human-type tubercle bacilli, strain Nakano, were mixed with two kinds of egg-yolk solution, a 37 per cent and a 3 per cent, at the rate of 0.01 mg per 0.5 ml solution. The bacilli-yolk mixtures were then inoculated subcutaneously into 2 groups of 6 guinea-pigs each, respectively, each animal receiving 0.5 ml mixture. At the same time, a third, control group of an equal number of guinea-pigs received 0.01 gm of bacilli suspended in 0.5 ml of distilled water without yolk. After inoculation, local lesions at the site of inoculation and tuberculin skin reactions secured with a hundredfold Old Tuberculin (Sauton culure) were observed and recorded weekly. Nine weeks after inoculation, all the animals were killed 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 from the spleens of all the animals.

The experiment led to the following results:

1. Egg-yolk enhances local lesions at the site of inoculation of tubercle bacilli. Within the extent of the present experiment, however, the difference in concentration of yolk seems to have no direct relation to the intensity of local lesions.

2. Egg-yolk exalts tuberculin allergy induced in animal by inoculation of tubercle bacilli.

3. In accordance with the above findings, egg-yolk enhances tuberculous lesions in the visceral organs and the lymph nodes. (It was worth noticing that, though not so intense, there was found histologically exudative disease in the lungs of animals injected with yolk of a high concentration).

4. In animals injected with yolk of a high concentration, the proliferation of the bacilli inoculated is markedly promoted.

5. The mechanism of the exalting action of egg-yolk on the virulence of the tubercle bacillus is not clarified as yet.

Drug-Resistance of Tubercle Bacilli Isolated from Pulmonary Tuberculous Lesions (I).

Yoshitame NAGAYAMA and Koji MOCHIZUKI

Tubercle bacilli were isolated from 136 positions of pulmonary fragments resected from 36 patients who had been treated with streptomycin, PAS and nitrazide, alone or in combination. The strains of bacilli were then examined for resistance to the chemotherapeutic agents just mentioned in order to obtain information about the ralationships between the characteristics or positions of lesions and the development of resistance of bacilli, between the degrees of resistance of bacilli in lesions of various forms and the chemotherapeutics used and about the difference in resistance between the bacilli detected in sputa and those submerged in lesions. The results obtained are as follows:

1. In the same patients considerable differences in resistance were noted among strains of tubercle bacilli isolated from lesions of differennt histopathological forms or from different positions of the same lesion.

2. The rate of appearance of resistance and the degree of resitance were found to be exceedingly high in materials removed from cavities, followed by hose from disseminated lesions. The rates were the lowest in caseous lesions.

3. In patients who had previously been treated by streptomycin alone, high resistance to the same agent was found in tubercle bacilli isolated from cavities.

4. The resistance of tubercle bacilli in cavities to streptomycin was found proportional to the amount of streptomycin used.

5. The resistance of tubercle bacilli in sputa almost reflected that in cavities.

Comparative Study on the Three Methods for Staining Tubercle Bacilli, Ziehl-Neelsen's, Alexander's and Hallberg's and Examination of Tubercle Bacilli in Human Pulmonary Lesions Using These Three Staining Methods in Parallel with the Culture Method.

Koji MOCHIZUKI and Yoshitame NAGAYAMA

Fifty-five samples of sputum obtained from patients with pulmonary tuberculosis and 331 resected pulmonary fragments were first submitted to a comparative study of the different three staining methods as mentioned in the title. Later on, on the basis of the findings obtained, a study was made of the relationship of the morphology, staining ability and reproducibility of tubercle bacilli to the types and characteristics of tuberclous lesions, using in parallel the culture method. The following are the conclusions drawn from the results of the study.

1. The three methods showed no significant difference in the positivity in detecting tubercle bacilli in the materials used. As for the ratio of the total number of bacilli detected in the same number of materials, that of Hallberg's method was the highest, followed by that of Alexander's, as far as the samples of sputum were concerned. However, for the materials removed from pulmonary lesions, the ratio was lined up in the descending order: Alexander's, Hallberg's and Ziehl-Neelsen's.

2. Both stain-and culture-positive cases were exclusively predominant for materials containing open cavities. Moreover, in these materials, viable bacilli were found to be remarkably numerous. On the contrary, stain-positive but culture-negative cases were noticed considerably frequent for materials with closed cavities and for those with caseous lesions. In these materials, viable bacilli were found only in a small number.

3. A distinct correlation was found between the morphology and the reproducibilty of tubercle bacilli. As the bacilli transform themselves from the normal rod form step by step into the clump, thin-thread and granular, dust forms as detected by Alexander's method, the quantitative culture becomes more and more scanty.

Study on the Morphology of the Tubercle Bacillus
I. Influence of ultra-violet irradiation.

Norio TSUKIORI

Human type and avian type tubercle bacilli and BCG were inoculated homogeneously on collodion-film-culture media, which were immediately exposed to ultra-violet irradiation during different periods of time varying from 10 seocnds to 2 minutes and incubated at 37°C. After adequate periods of incubation, changes in the morphology and in the staining property of the bacilli were comparatively examined by means of the electron microscope as well as of the light microscope (Ziehl-Neelsen's and Fonte's staining methods). The results were as follows:

1. Viewed from their position and number in the bacillary cells, no definite correlation was found between the so-called Much's granula and the granula non-transparent to electron (NT-

body, Knaysi's A body).

2. Under the influence of ultra-violet irradiation, the cytoplasm of the cells underwent considerable changes and its transparency to electron augmented, the cells exhibiting the so-called ghost forms. As the transparency to electron augmented, the acido-resistance diminished.

Study of Tubercle Bacilli Naturally Resistant to Nitrazide (II)

Akihiko KUZE

Comparative study was made on strains of tubercle bacilli naturally resistant to nitrazide of as high a concentration as 100 γ per ml and their parent sensitive strains. The experiments led to the following results:

1. No difference was noted in the intensity of tuberculin allergy between guinea pigs inoculated with resistant strains and those inoculated parent sensitive ones.

2. The virulence to guinea pigs of 2 of the 3 resistant strains studied was found to be considerably weaker than that of their parent sensitive strains. While that of another one was almost equal to its parent strain.

3. No difference was found in the activity of lactate-dehydrogenase between the two kinds of bacilli, sensitive and resistant.

4. Entire lack of catalase was noted in naturally resistant tubercle bacilli, but lack of catalase did not reflect diminution nor loss of virulence.

5. In the staining abilty and the morphology as observed by the light microscope, no difference was noted between the two sorts of bacilli, sensitive and resistant.

6. The electron-microphotographs of the two resistant strains lacking entirely catalase and far less virulent than their parent sensitive strains exhibited a feature as if the bacilli lacked the surrounding slime substance, their contour being clear-cut. While the another resistant strain similarly lacking catalase but as virulent as its parent sensitive strain presented the same electron-microscopical feature as its parent sensitive strain.

7. When mixtures of resistant and sensitive strains were repeatedly transplanted and cultured on media without nitrazide, the ratio of numbers of resistant colonies to those of sensitive colonies rapidly decrease.

Studies on the Generation Time of Mycobacterium Tuberculosis
I. Determination by the Use of Small Inoculum Technique and of the Micro-Kjeldahl Nitrogen Determination.

Yoshihito SHIMMYO, Toshiwo YOKOI and Takawo SHIDA

The generation time of BCG as estimated by the small inoculum technique was 21.7, 19.2 and 15.6 respectively in three experiments separately performed, while that estimated by the micro-Kjeldahl nitrogen determination, using 4 different amounts of inoculum, was 4.04, 3.88, 2.87 and 2.84 days respectively.

Studies on the Generation Time of Mycobacterium Tuberculosis II. Determination by Turbidity of Cultures of Bacillary Suspensions.

Yoshihito SHIMMYO, Takawo SHIDA and Toshiwo YOKOI

Kirchner's media containing tween 80 at the rate of 0.05 per cent were inoculated with 0.1, 0.05 and 0.01 mg of BCG per ml respectively and incubated at 37°C. They were then examined of their turbidity every 24 hours. From the values thus obtained the generation time of BCG was calculated. It was approximately 48 hours.

Studies on the Generation Time of Mycobacterium Tuberculosis III. Determination in Vivo.

Yoshihito SHIMMYO

Sixty mice of the dd strain were divided into six equal groups. They were infected by the intravenous route with doses of tubercle bacilli which varied from group to group.

The generation time of tubercle bacilli inoculated in mice of this strain was calculated from the median survival times of the animals of each group using the method of Litchfield.

The generation time of the human type tubercle bacillus, Nakano strain, was found to be 26.1 days.

Analytical Studies on the Koch Phenomenon, with Special Reference to the Relationship between Allergy and Immunity in Tuberculosis

By

Toru ŌHARA, Takashi IKEHATA and Tomowo OGITA

The mode of appearance of Koch's phenomenon has been analysed, taking into consideration the effects of various quantitaive combinations of antigen and antibody and observing the subsequent outcome of the local reactions. Thus exact information on the relation between allergy and immunity in tuberculosis has been obtained. In this analytical study, it has been clearly shown that allergy and immunity run parallel with each other in animals with low antibody titer, while being dissociated from each other in animals with high antibody titer. In other words, immunity increases with the increase of antibody titer, whereas the reaction of Koch's phenomenon decreases in intensity, showing its strongest reaction in animals with moderate antibody titer. The fact that Koch's phenomenon does not make its appearance in animals with very low antibody titer can be attributed to the state of normergy of the animals, and the same fact observed in animals with high antibody titer can be ascribed to the animals' having already passed through the state of allergy into the state of complete immunity. This indicates that allergy is an intermediate process which manifests itself in the biological process from normergy to complete immunity. Therefore, we consider that, in tuberculosis, both allergy and immunity are based on one and the same mechanism, allergy being a pre-stadium or an incomplete expression of immunity.

Studies on the Antitubercular Compounds. X. Condensation Products of Aldehydes and Acid Hydrazides of Pyridin Group.

Shichiro KAKIMOTO and Ken-ichi YAMAMOTO

Nine condensation products of isomeric three aldehydes and three acid hydrazides of pyridin group were prepared and examined whether it should have the tuberculostatic activity against virulent human tubercle bacillus, strain H 37 Rv, on Kirchner's medium containing 10 per cent of bovine sera. The most active compounds were condensation products of isonicotinic acid hydrazide with aldehydes and stronger than isonicotinic acid hydrazide in vitro.

The Urine Quotient and Tuberculosis
I. The urine quotient as a means for measuring the metabolic function and its application to thoracic surgery.

Osamu NISHIKAZE and Tokuji NOZAKI

This report deals with the results of the application of the urine quotient O/K_3 to post-operative chest disease patients and post-operative abdominal cancer patients.

1) O/K_3 value of tuberculosis patients prior to operation stood at 37.1 ± 16.6, whereas O/K_3 value of cancer patients prior to operation stood at 136.5 ± 42.4.

Statistically, both cases showed a high value as compared to the normal value 21.9 ± 4.1. Moreover, a remarkable difference was seen between tuberculosis patients and cancer patients.

2) Daily O/K_3 values measured in post-operative tuberculosis patients were as follows:

1 st day after operation	111.6 ± 47.8,	2 nd day	103.8 ± 54.4,
3 rd day	84.8 ± 54.2,	4 th day	45.9 ± 31.0,
5 th day	40.3 ± 8.7,	6 th day	35.9 ± 12.0,
7 th day	34.1 ± 15.9,	9 th day	43.0 ± 29.5,
11 th day	43.4 ± 33.2	and 13 th day	43.7 ± 20.0.

While daily O/K_3 values in post-operative cancer patients were as follows:

1 st day	238.0 ± 190.4,	2 nd day	151.3 ± 15.0,
3 rd day	140.7 ± 88.1,	4 th day	145.0 ± 114.5,
5 th day	95.4 ± 17.1,	6 th day	153.7 ± 90.6,
7 th day	216.3 ± 172.5,	9 th day	108.6 ± 30.2,
11 th day	82.9 ± 30.0	13 th day	82.9 ± 30.0
and 15 th day	66.6 ± 20.1.		

As seen above, a remarkable difference was noticed statistically in all individual figures as compared to the normal value. Moreover, it was worthy of notice that a significant difference was observed between both cases from the 1st to 13 th day after operation.

Immunological Study of Sera of Tuberculous Animals
II. Paper-Electrophoretic Study of Sera of Tuberculous Rabbits

Harue OKUYAMA and Kazuo MORIKAWA

One group of rabbits was first injected with BCG and then challenged with virulent tubercle bacilli or desensitized with old tuberculin. Another group of rabbits was infected with virulent bacilli without preparing inoculation with BCG. At different phases of the experiments sera were

obtained from the animals and submitted to paper-electrophoretic investigation.

The experiments led to the following conclusions:

1) In comparison of these analyical values with those obtained by the Tiselius method as reported in the previous paper, some quantitative differences were observed: with the same materials, when measured by paper electrophoresis serum albumin and α-globulin were greater, while β- and γ-globulin were smaller in quantity than by the Tiselius method of electrophoresis.

2) In rabbits inoculated with BCG or with virulent bacilli, a remarkable increase in the quantity of serum γ-globulin and a slight increase of α-globulin were noticed.

3) Both challenge with virulent bacilli and desensitization with old tuberculin produced a ramarkable decrease in the quantity of γ-and α-globulin 6 and 10 hours after treatment.

4) Sera of tuberculous rabbits showed a decrease in γ-and α-globulin when absorbed *in vitro* with old tuberculin, water extract of tubercle bacilli or viable virulent bacilli. Particularly, absorption with viable bacilli caused a remarkable decrease in γ-globulin.

5) It is clear from this study that sera from tuberculous animals are characterized by the increase in quantity of γ-and α-globulin, as previously shown in the electrophoretic study by the Tiselius method. The role of these components in immunity in tuberculosis was discussed.

Immunological Study of Sera of Tuberculous Animals
III. Passive Transfer with the Serum γ-Globulin of Tuberculous Rabbits

Harue OKUYAMA and Kazuo MORIKAWA

From the sera of tuberculous rabbits protein fractions were prepared with alcohol by Cohn's method and by the paper-electrophoretic method. By the use of these preparations passive transfer of tuberculin sensitivity was attempted in the rabbit skin by the Prausnitz-Küstner method.

These experiments led to the following conclusions:

1) The solution of the γ-globulin fraction showed the precipitin reaction toward old tuberculin and tuberculoprotein.

2) In the cutaneous sites where lower dilutions of γ-globulin had previously been injected, an intense reaction ocurred following subsequent injection with old tuberculin or PPD. Moreover, tuberculin sensitivity evoked by γ-globulin was distinctly intensfied, when injected mixed with alminium hydroxide.

3) The tuberculin reaction in the skin where γ-globulin was previously injected was histologically compared with the same reaction in the skin where peritoneal cells of tuberculous rabbits were injected. In both cases polymorphonuclears were predominant in the early stage of the reaction. However, predominance of mononuclears in the later stage of the reaction was more apparent in the sites where tuberculous cells were inoculated than in those where γ-globulin was injected.

4) Whether the tuberculin sensitivity thus transfered with the serum γ-globulin fraction is of delayed type or not remains yet to be studied.