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Abstracts of "Tuberculosis Research"

Vol. 6, 1956.

Studies on the Antitubercular Compounds (XII) Effect on Antitubercular Activity of Acid Hydrazide by the Condensation with Aldehyde

Shichiro KAKIMOTO and Ken-ichi YAMAMOTO

In a previous paper, it was reported that *in vitro* tuberculostatic activity of isonicotinic hydrazide was promoted by condensation with pyridin aldehyde.

But the activity of acid hydrazide has not been increased by condensation with other aldehydes which might have affinity for tubercle bacilli. The compounds having free amino group are active on Kirchner's medium without serum, but not on that containing bovine serum at the rate of 10 per cent.

Tuberculosis and Urine Quotient

3. Urine quotient as a reliable criterion for measuring the systemic faculty of human bodies under various conditions and its clinical application to silicosis, with special reference to silico-tuberculosis

Toyoo KOBAYASHI

A statistical study of silicosis was made on two thousand-six hundred workers at the "S" coal mine in Hokkaido by means of roentgenography, while a clinical and biochemical study was conducted on the sixty four patients.

1) Statistical:

Though the statistical frequency of silicosis at the "S" coal mine was lower than that at metal mines, three exceptionally serious cases were found contrary to the general belief and it was further revealed that the said workers were strict colliers.

2) Clinic:

The data obtained by using Thorn's test and determining the so-called "capillary resistance" suggested that in silicotic patients, especially in silico-tuberculous cases, there is a remarkable decrease in the systemic reactivity depending on the hypofunction of the hypothalamo-hypophyseal system.

3) Metabolic:

i) O/K_4 (as the latest means of determining the general adaptation faculty of human bodies at given moments): in mild, moderate and severe cases of silicosis the ranges of O/K_4 value were 30 to 100, 50 to 200 and 70 to 500 respectively, whereas in the cases of silico-tuberculosis the respective ranges were 50 to 200, 100 to 800 and over 1,000. When the range exceeds 1,000, the O/K_4 value is considered as the infinit value (∞), in which case the severity is equal to that of the O/K_4 value in severe cases of cancer.

ii) Administration of adrenalin (3 mg.) and pituitrin (3 units) to silicotic patients showed a

remarkable rise in O/K_4 value in the severe cases with a simultaneous fall of urine excretion and chlorine excretion within the experimental period of three hours after administration, but not in the mild and moderate cases.

These results obtained from the above observations showed that there is a marked decrease in the adaptive reactivity of the severe silicotic patients, especially of the severe silico-tuberculous cases in the response to changes in their environments. In other words the data imply that the decrease in the adaptive faculty of the silicotic patients, especially of the silico-tuberculous cases which can be grasped by using a new method (Urine Quotient), is associated with the possible hypofunction of the hypothalamo-hypophyseal system, which results in disturbances of water-, salt- and protein-metabolism.

Morphological Study of *Mycobacterium Tuberculosis* II. On the Biological Significance of the Electron Non-Transparent Body

Norio TSUKIORI

Electron-microscopical and cytochemical study was made on microcultures of human tubercle bacilli grown on collodion film media from which the carbon and nitrogen sources had been removed at varying degrees.

Thus, the behavior of the electron non-transparent body was compared dynamically with that of the granules stained with various cytochemical techniques (Fontés, Janus-green B, Delafield's hematoxylin, Feulgen). The study led to the following conclusions:

1. Under varying conditions, the numerical distribution of the electron non-transparent bodies was proved to coincide well with that of the granules stained with Janus-green B.
2. Under favorable conditions, when the bacilli were actively multiplying, the stained granules as well as the non-transparent bodies were formal usually one by each of the two poles of the bacillus. On the contrary, under unfavorable conditions, only the Janus-green B stained granules exhibited almost the same distributional behavior as that of the non-transparent bodies.
3. The distributional behavior of the Feulgen-positive granules was found almost unchanged under all the experimental conditions, being always inconsistent with that of the non-transparent bodies.
4. On the basis of the facts just described, the present author considers the electron non-transparent body to be of mitochondria-like character (chondrioid).
5. Removal of the carbon source from the media had little influence on the morphology of the bacillus, while removal of the nitrogen source had a considerable influence on the fine structure, growth and acid-fastness of the bacillus. Especially, the influence of serum was the greatest.

Natural Infections with Drug-Resistant Tubercle Bacilli

Syun TANIWAKI

From June 1953 to November 1955, drug-resistance was tested in 127 hospital patients with pulmonary tuberculosis among whom 15 were found resistant against more than 10% of streptomycin and 1 against 17 of isoniazide.

Cases of streptomycin-resistance increased year by year: no case before 1954, 7.8 per cent in 1955 and 18.6 per cent in 1956.

Combined treatment with streptomycin, PAS and isoniazide proved favorable for these resistant patients clinically as well as roentgenologically, except for 2 cases in which pulmonary x-ray findings remained unchanged.

Systemic Response and Anti-Tuberculous Therapeutics

1. The effect of SM and INAH on formaline inflammations in rats

Hideyo ITAYA

This is the first report of a series of studies which are being conducted to find or otherwise to suggest a new approach to the synthesizing of anti-tuberculous therapeutics from an endocrinological stand point.

In this study the effect of SM and INAH on a non-specific inflammation was obtained in rats with the following results:

1) Both SM and INAH showed a definite antiphlogistic action on acute and chronic formaline inflammations.

2) The administration of INAH caused a decrease in the cholesterol level of the adrenal gland through indirect stimulation of the hypophysis, whereas in the case of SM no changes were observed.

In other words it is emphasized from an endocrinological view-point that SM has less deversive effect as compared to INAH.

Studies on the Mixed Cultivation of Acid-Fast Bacilli with *E. coli*, *Candida Albicans*, *Staphylococcus*, and *Proteus x 19*

Toshio YOKOI

Four strains of virulent human tubercle bacilli, two strains of attenuated and avirulent human tubercle bacilli (Imamura and H37Ra), BCG, four strains of avian bacilli, and eight strains of nonpathogenic acid-fast bacilli were cultured on Sauton media mixed with *E. coli*, *staphylococcus aureus*, *Proteus x 19*, and *candida albicans* (*C. alb.*) respectively.

This was done for the purpose of observing cultural changes in the mixed organisms following different metabolic phases of the tubercle bacilli.

Especially for *E. coli* and *C. alb.*, the influence of tuberculin on their multiplication was investigated.

1) Incubation with *E. coli*:

The growth of the human tubercle bacilli inoculated with *E. coli* was clearly suppressed, while the multiplication of *E. coli* seemed to be slightly inferior to that of the control on Sauton media.

Regarding the growth of the avian bacilli and the nonpathogenic acid-fast bacilli incubated with *E. coli*, that of each of the mixed organisms was similar to that of the control on Sauton media.

2) Incubation with *C. alb.*:

The growth of the tubercle bacilli cultured with *C. alb.* seemed to be more or less inhibited, while the multiplication of *C. alb.* was markedly promoted, despite of the fact that *C. alb.* hardly multiplies on Sauton media.

The growth of the avian bacilli or the nonpathogenic acid-fast bacilli incubated with *C. alb.* was similar to that of the control on Sauton media, while the multiplication of *C. alb.* hardly took place in this case.

3) Difference in the growth between the virulent human tubercle bacilli and the attenuated human tubercle bacilli, BCG, in the mixed cultures :

No difference was observed in the growth between the virulent human tubercle bacilli on the one hand and BCG and the attenuated tubercle bacilli used on the other, the multiplication of *E. coli* cultured with three organisms being similarly the same, regardless of the virulence of the tubercle bacilli used.

4) Incubation with *Staphylococcus* or *Proteus* :

The growth of the acid-fast bacilli, *Staphylococcus* and *Proteus* was similar to that of their single culture.

5) The influence of tuberculin on the multiplication of *C. alb.* and *E. coli* :

When tuberculin was added to Sauton media, it promoted the multiplication of *C. alb.* either in a high or in a low concentration.

While, the multiplication of *E. coli* was promoted only by the addition of a high concentration of tuberculin and that at the early stage of incubation.

On the Antigenic Activity of Old Tuberculin after Successive Absorption with Sheep Red Cells

T. IMAI, M. ITAKURA, K. TAKAHASHI and T. NUMATA.

Three kinds of Old Tuberculin was absorbed with sheep red cells successively 14 times. After treatment, every sample of sensitized cells thus prepared was resuspended and tested for haem-agglutination and hemolysis.

In addition, the complement fixation reaction by the unit of 50 per cent hemolysis was carried out for the three kinds of Old Tuberculin before absorption and for the supernatant fluids after each absorption.

The experiment led to the following results.

1) The antigenic titre of Old Tuberculin in agglutination and hemolytic reaction gradually decreased with the repetition of absorption and finally came to zero, contrary to the data reported by Rheins and Thurston.

2) There was no difference in antigenic activity for complement fixation between the *original* Old Tuberculins before absorption and the ones after absorption 14 times.

It follows from these data that the complement-fixing antigen in Old Tuberculin can not be absorbed to sheep red cells.

Experimental Studies on the Biological Properties of Extracted Cells of *Myc. tuberculosis*

Shigetoshi TAKAGI

Biological properties of the following three kinds of extracted cell residues of tubercle bacilli were studied patho-histologically :

(1) Living human tubercle bacilli extracted with acetone (BA), (2) cells of BA extracted with methanol and chloroform (RC), and (3) cells of RC extracted with one per cent HCl-alcohol (RHA).

The extraction was carried out at 45°C.

These residues were injected intravenously to normal or BCG-immunized rabbits. After injection the animals were sacrificed at different intervals, and their visceral organs were examined. The results were as follows:

1. In normal animals BA produced a remarkable productive inflammation. The reaction was gradually intensified until 28 days after injection, accompanying a marked exudative change. On the contrary in the animals inoculated with BCG a striking exudative inflammation occurred from the first day after injection with BA, and from the 3rd day epithelioid cell tubercles were formed.
2. In normal animals RC was slightly weaker in pathogenic power than BA, but in immunized animals it produced much intenser lesions.
3. RHA had only slight effect on normal animals, but it produced a marked change in immunized animals from the early days after injection.
4. No qualitative difference was observed among the lesions produced by those 3 kinds of cells in the immunized animals.
5. The role of the chemical components of *Myc. tuberculosis* in the formation of tuberculous lesions was discussed.

Immunological Study of Sera of Tuberculous Animals

IV. Role of Complement and Serum Protein Fractions in Allergic Reactions

H. OKUYAMA, H. TOKITA, S. TAKAGI, & T. HASHIMOTO

In order to study the quantitative change in complement (C') and in the serum protein fractions in the course of allergic reactions, experiments were undertaken. The results obtained were as follows:

1. In sera of guinea pigs a temporary increase in C' titer 1 or 2 weeks after injection of ovalbumin or BCG. The injection caused also a gradual increase in the quantity of the serum γ -globulin.
2. In guinea pigs sensitized with ovalbumin, striking C' decline occurred from the 30th minute to 10th hour after reinjection. The quantity of the serum γ -globulin temporarily increased at the 30th minute, then it decreased remarkably.
3. In case of anaphylactic shock with ovalbumin, C' titer first increased, while it decreased after death.
4. In guinea pigs inoculated with BCG a remarkable C' decline occurred 3 and 10 hours after injection of old tuberculin, and C' titer continued its low value until 24 hours. On the other hand the quantity of α -globulin was at the lowest at the 3rd hour, and that of γ -globulin at the 10th hour.
5. From this study the complement decline was proved to have no direct relation to the decrease in the quantity of the serum protein fractions, however both complement and serum protein were found to play a great role in allergic reactions.