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Abstracts of "Tuberculosis Research"
Vol. 8, 1957.

Studies on the Antitubercular Compounds (XIV)
Cross Resistance among Derivatives of Pyridin

S. Kakimoto and K. Yamamoto

Several kinds of pyridin derivatives, as shown in table 1, were prepared and then resistant strains to each compound were made or selected.

These compounds contain a pyridin nucleus as the affinitive group for tubercle bacillus. They contain, respectively, acid hydrazide, thioamide and thiosemicarbazone as the toxic group. The fact that tubercle bacilli are resistant to a certain compound is supported to be that they are against the toxic group of the compound and not against its affinitive group.

Influence of Egg-Yolk on the Virulence of Mycobacterium Tuberculosis
IV. Influence of egg-yolk on the in vivo multiplication of BCG and its immunizing effect.

K. Yamamoto, A. Hagiwara and M. Itoh

It was shown in a previous paper that tuberculin allergy and immunity produced in guinea pigs following vaccination with BCG suspended in egg-yolk solution was about hundred times as high as the same amount of BCG inoculated in suspension with distilled water. This finding was supposed to be due to the promoted multiplication or the accelerated distribution of BCG incorporated in egg-yolk in the tissues of the vaccinated animals.

In order to verify this supposition, efforts were made to pursue the survival of BCG in the vaccinated animals and to estimated the efficacy of vaccination of BCG mixed with egg-yolk.

Guinea pigs were divided into three groups of an equal number, one receiving subcutaneously 0.01 mg of BCG suspended in distilled water, the other the same dose of BCG suspended in 50 per cent egg-yolk solution and the third 1 mg suspended in distilled water. Three, 7, 14, 21 and 35 days after vaccination, three animals were sacrificed of each group. The multiplication and survival of the bacilli vaccinated were followed up quantitative culture on local lesions at the site of vaccination, its satellite lymph nodes, the spleen, lung and liver. Three and five weeks after vaccination, tuberculin skin reaction were examined. Five weeks after vaccination, the remainder of the animals, together with the control animals, were challenged subcutaneously by the injection of 0.005 mg of virulent, human-type tubercle bacilli, strain Nakano. After inoculation all the animals were observed according to the same method as described in the previous paper.

The experiments led to the following results.
1. No appreciable difference was observed throughout the experiment in the viable unit of BCG recovered from the spleen, lung and liver between the groups with or without egg-yolk. However in
the animals with egg-yolk, increased multiplication of BCG was found at the site of vaccination from three weeks after vaccination, while in the satellite lymph nodes it began shortly after vaccination and lasted until 3 weeks.

2. The animals vaccinated with BCG suspended in egg-yolk solution showed against the challenge infection with virulent tubercle bacilli a resistance much greater than the animals vaccinated with BCG suspended in distilled water, their immunity being about hundred times as high as the same amount of BCG suspended in distilled water.

3. The present result was explained by the assumption that egg-yolk accelerated the multiplication of BCG in the lesions at the site of inoculation and in the satellite lymph node of the vaccinated animal and thereafter the proliferated BCG therein stimulated the production of immunity against tuberculosis.

Effect of Antituberculous Chemotherapeutics on the Morphology, Viability, Acid-fastness, and Cytochemical Staining Properties of Tubercle Bacilli

J. ARIMA, N. TSUKIOBI and Y. TAKAHASHI

Comparative study was made on the influence of Streptomycin (SM), isonicotinic acid hydrazide (INAH) and para-aminosalicylic acid (PAS) upon the morphology, viability, acid-fastness and cytochemical staining properties of human type tubercle bacilli. The study led to the following conclusions:

1. Both for SM and INAH, the bactericidal action was observed toward tubercle bacilli (H₃₇Rv) growing in Dubos-Davis liquid media, while for PAS, little bactericidal action was noticed.

2. The electron-microscopical pictures of the bacilli following contact with SM revealed augmentation in the electron-transparency of cytoplasm, disappearance of the electron non-transparent body, and lysis of bacillary bodies. Almost the same findings were also obtained for INAH, thus suggesting lack of a qualitative difference in the morphological changes of bacilli caused by SM and INAH.

3. A finding was obtained showing the existence of the phase in which little morphological change occurred in spite of the disappearance of the viability of bacilli following contact with SM and INAH.

4. Disappearance of the acid-fastness of bacilli were induced by the action of INAH as well as SM.

5. Drug resistant bacilli developed, both in cases of SM and of INAH, from 1 to 3 weeks after addition of the drugs into the liquid culture, presenting no morphological structures different from those of drug sensitive normal bacilli.

6. The behavior of the electron non-transparent body was proved not necessarily to coincide with that of the granules stained with various cytochemical techniques, such as Feulgen reaction, Janus-green B, and Toluidine-blue.

Clinical Study of the Phosphatide-Hemagglutination Reaction in Tuberculosis

(Y. TAKAHASHI and H. FUKAE)

In respect of the hemagglutination reaction, comparison was made using sera from 75 tuberculous
patients and from 26 tuberculin-positive, normal adults, between tuberculin protein, tuberculin polysaccharide and bacillary phosphatide, the former two isolated from a sample of unheated Sauton tuberculin and the latter from a Sauton culture of the same human tubercle bacilli strain Nakano.

Sheep erythrocytes were sensitized with each one of the antigens and put into contact with serial dilutions of the sera. The procedure and the reading of the data were made according to the usual method.

For tuberculin protein and polysaccharide, all the sera, tuberculous and normal, presented positive reactions, giving no marked difference which may be of service to diagnosis of the evolution of the disease, except that the tuberculous sera reacted more strongly than the normal sera.

On the contrary, for phosphatide, while all the tuberculous patients, except 2 slight cases, gave positive reactions, 17 of 26 normal adults (65%) were completely negative, the other 9 adults showing positive reactions only slightly. In addition, of the tuberculous patients, the serious cases reacted far more intensely than the slight cases.

Biological Significance of the Electron Non-transparent Body of the Tubercle Bacillus

Y. TAKAHASHI, T. YAMASHITA, N. TSUKIORI and S. TAKAHASHI

The electron non-transparent body of the tubercle bacillus (A body after G. Knaysi et al) is at present considered either as nucleus or as mitochondrium-like body or as metaphosphate body or further as volutin (complex of Ca-metaphosphate-nucleic acid).

Electronmicroscopical and cytochemical experiments which were carried out to determine its real biological character did not furnish any data which could support any one of the foregoing four hypotheses, while evidence was obtained, suggesting that the electron dense body in question is rather a reserve material mainly of lipoidal and polysaccharine nature.

As cytochemical technics, use was made of the Feulgen nucleal reaction for nucleus, janus-green-B stain for mitochondrium, toluidine-blue and lead-sulfide stains for metaphosphate, the Lillie method for polysaccharide and sudan black-B stain for lipoidal substance. In addition, the sites of reduction of potassium tellulate in the interior of the bacillary cells were observed on bacilli grown on Sauton media to which potassium tellulate had been added.

Before staining, the bacilli (avian mycobacteria 4 days old on Sauton media) were treated, in case of need, with acetone or 5 percent HCl, and were stained as they were in suspension. On finishing staining, a droplet of the suspension was brought onto the surface of a copper mesh provided with thin collodion film and dried. Thus, the light- and electron-microscopical findings were compared on the same bacilli stained.

Pretreatment with acetone or HCl almost completely deprived the bacilli of their A bodies, while Feulgen granules remained as they were.

Neither janus-green-B granules nor toluidine-blue granules did always correspond to A bodies.

The same was almost true of lead-sulfide granules: of these, some did not correspond to A bodies, indicating that metaphosphate exist also in the cytoplasm besides their environment.

Crystals of reduced tellilium were found attached to A bodies, thus increasing their dimensions. They were also observed sometimes locally sprinkled, sometimes totally disseminated in the cytoplasm.

Polysacharide granules, when stained, corresponded well to the opaque, cytoplasmic coagulations
reminiscent of the presence of A bodies.
Likewise, sudan-black-B granules could be observed fairly corresponded to A bodies.

Studies on the Precipitin Reaction of Tuberculous Human Sera
by the Use of Toxic Lipid (Cord Factor)
Extracted from Tubercle Bacilli

S. ISHIYAMA, E. SUMIKAWA and Y. SHIMMYO

Sera obtained from tuberculous patients were tested for their precipitin reactions towards CF-antigen prepared from lipid material which is considered to contain so-called cord factor. Results obtained were as follows:

1) CF-antigen proved to have an in vitro antigenicity comparable to that of H2 antigen (water extract of tubercle bacilli) which has previously proved in our laboratory to be an excellent antigen for the titration of tuberculous sera.
2) CF-antigen seemed to be more closely related to the gravity of tuberculosis than does H2-antigen.
3) All sera obtained from healthy persons with one exception and all sera obtained from patients suffering from serious diseases other than tuberculosis gave no positive reaction towards CF-antigen.

Amino Acid Composition of the Protein of
Tubercle Bacilli and of Tuberculin

M. ITAKURA

By the use of paper chromatography, the protein of tubercle bacilli proved to be composed of the following 16 amino acid components: serine, asparatic acid, glutamic acid, glycine, alanine, valine, leucine, phenylalanine, threonine, proline, cystine, histidine, arginine, lysine, methionine and tyrosine. Besides, tryptophane was found in the protein of tubercle bacilli when bacilli were extracted with alkali instead of HCl.

The amino acid composition of tuberculin was found to be the same as that of tubercle bacilli except that tyrosine could not be demonstrated.

Studies on the Metabolism of Tubercle Bacilli
II. The Effect of TCA Cycle Intermediates and Precursors
on the Respiration of Mycobacteria

K. TAKAHASHI

Krebs' TCA cycle intermediates were examined for their effects on the respiration of M. avium (strain Takeo), M. phlei, M. tuberculosis var. hominis (strain H 37 Rv) and BCG cultivated in Kirchner's media for one to five weeks respectively. Results obtained were as follows:

1) No evidence was found that the four strains differ essentially from each other in the utilization of the Krebs' TCA cycle intermediates and precursors for their respiration.
2) Lactate, pyruvate, glycerol and glucose had a stimulatory effect on the respiration of my-
cobacteria tested, this effect being parallel with their concentrations.

3) Acetic acid in low concentration was stimulatory on the oxygen uptake of mycobacteria, but in higher concentration it was inhibitory.

4) Fumarate and -ketogultarate showed a slight stimulatory effect on the respiration of younger cultures of mycobacteria tested, but showed no effect on that of older cultures, whereas l-malate was stimulatory only slightly on older cultures of mycobacteria.

5) Cis-aconitate, succinate and citrate had in no case a stimulatory effect on the respiration. Instead, citrate in high concentration inhibited the respiration.

6) Optimal hydrogen ion contentrations of the substrates were found in the acidic side in the earlier stage of cultivation, but they turned to the alkaline side in the later stage of cultivation.

Studies on the Tuberculin Allergy in Mice

Y. SHIMMYO, K. TAKAHASHI, T. HASHIMOTO and T. KOBAYASHI

Tuberculous mice, which have hitherto been considered as naturally anergic to tuberculin showed positive tuberculin reaction, when old tuberculin was injected into their footpad. This footpad reaction was of delayed type, being strongest during the period of 24 to 48 hours after injection of tuberculin. Footpads of mice injected with tuberculin showed erythema and diffuse swelling. Histo-pathological examination revealed that this reaction was quite identical to that observed in guinea pigs or rabbits. The minority of mice turned allergic to tuberculin about 4 weeks after infection with tubercle bacilli, but the majority of them did so after about 7 weeks after infection. This footpad reaction showed a tendency to weaken from the 10th week on.

Tuberculosis and Urine Quotient

5. A critical study on Urine Quotients (O/K, O/K₂, especially O/K₃) as criteria for measuring the vitality of human bodies, with reference to cancerous, especially tuberculous patients.

K. IWATA

In 1949, Nishikaze of our research group reported that his O: K ratio could be adopted as a criterion for measuring the degree of fatigue. Later on, a new approach was proposed for the study of fatigue as a part of a series of studies on fatigue.

In the present paper, comparative studies were made on O/K, O/K₂, especially O/K₃ in tuberculous patients and postoperative chest surgery patients together with cancerous patients in advanced stages.

The results were as follows.

1) It was revealed that no direct correlation existed between the urine quotients, especially the new quotient O/K₃ and urine volumes, chlorine values in urine together with its concentration and/or urine phosphor values together with its concentration and urine pH measured at the same time and that the O/K₃ value increased in parallel with the degree of advancement of stress on the living body. Namely, while the O/K₃ value of normal adults (control) was 21.9±4.08, it ranged from 150 to 25 in tuberculous patients, and from 230 to 70 (highly significant) in advanced cancerous patients. This indicates that the O/K₃ is superior to O/K, and O/K₂ being highly satisfactory as a means for vitality measurement in humans.
2) It was noted that the fluctuation of the $K_2$ value showed no direct correlation with that of the other biological substances or reactions.

3) It was also noted that the iodate number values, $(K, K_2, \text{etc.})$ especially the $K_2$ value decreased approximately in parallel with the decrease in the vitality of the living body.

**Tuberculosis and Urine Quotient**

6. A critical study on the urine quotient, $O/K_3$, as a means for measuring the vitality of human bodies, with a special reference to pulmonary tuberculosis patients after operation.

D. UETAKE

With the advance in the field of anesthesiology and following the introduction of antibiotics, the surgical treatment of tuberculosis has made a remarkable advance with a definite drop in the mortality rate from chest surgery.

However, it is noted that this very scope and range of application is limited, depending upon the fact that qualitatively definite basic determination have not been made as regards blood transfusion and infusion solutions. In other words, the basic condition to be universally accepted have not been determined as yet.

It may be said that hitherto success or failure in operations was generally determined by the death rate in patients operated on. However, in order to determine the basic preconditions for blood transfusion and supplementary fluids as mentioned above, death rates alone are insufficient and it is considered as imperative that the application of a method that will enable the grasping of the vitality of the living body be made objectively. However at present the absence of such a method is surprising.

Hence as indicated in the title, the application of Nishikaze's Urine Quotient is suggested (Vitality Criterion).

With the above in mind, comparative studies were made on 40 chest surgery cases, taking special attention on pre- and post-operative fluctuations of the urine quotient.

1. In 16 cases in which the $O/K_3$ value rose remarkably after operation, it was shown that urine protein increased remarkably with low values in urine volume and chlorine value, indicating hyper- or hypo-function of the adrenal gland.

2. It was noted that the fluctuations in the $O/K_3$ value were not influenced directly by the type of operation. It was, however, seen that the $O/K_4$ values were not so high in the group in which closed respiratory anesthetics were used and in the groups in which a comparatively large volume of blood was transfused and/or a comparatively large volume of infusion solution, 5% Glucose, not Ringers' solution, was used in the post-operative period.

3. The $O/K_3$ values were higher in cases in which lower values in the breathing capacity and the duration of respiratory standstill were seen.

4. It was noted that the $O/K_4$ values were higher in cases in which body temperature rose and especially in cases in which pulse rate increased in the post-operative period.

From the above results, it may be said that the quotient method ($O/K_3$), as reported previously by Nishikaze, et al., is an almost ideal fatigue criterion, having no direct correlation with given organ function or given metabolic functional system in the living body.

Thus, it may be said at least that the present $O/K_3$ method can well be utilized in the determina-
tion of the basic quantitative and qualitative condition of transfusion and supplementary fluids as mentioned above.

Experimental Studies on the Biological Properties of Chemically Extracted Cell Residues of *Mycobacterium tuberculosis*

S. TAKAGI

By the use of the following extracted cell residues of tubercle bacilli, the role of the bacillary components in the histogenesis of tuberculous lesions was studied: 1) living human tubercle bacilli extracted with acetone (BA), 2) cells of BA extracted with methanol and chloroform, successively (RC), and 3) cells of RC extracted with one per cent HCl-alcohol (RHA).

The experiments undertaken were composed of 3 parts: 1) The action of the residues on normal rabbits, 2) the action of the residues on rabbits inoculated with BCG, and 3) the action of the residues as immunogens against virulent challenge in rabbits and guinea pigs. The studies led to the following conclusions.

BA produced lesions similar to those provoked by the usual heat-killed bacilli in normal and BCG-inoculated animals. Animals injected with BA acquired an immunity against virulent challenge.

RC, a cell residue in which no free lipid was detectable, produced almost the same changes as did BA in normal and BCG-inoculated animals. Moreover, RC was found to be a good immunogen against virulent challenge.

RHA, a cell residue which had no bound lipid in its cellular component and had no acid-fastness, produced only slight tubercular lesion in normal animals. However, it could provoke a severe allergic reaction in BCG-inoculated animals. RHA possessed almost no immunogenicity against virulent challenge under the experimental conditions.

From these results the role of the bacillary components in the histogenesis of tuberculous lesion and in the development of acquired immunity was discussed.

Pathological Study on Delayed Shock

H. TOKITA

In order to study the pathology of delayed shock the following anatomical experiments were undertaken in the rabbit: 1) necropsy studies of delayed shock resulting from the intravenous superinfection of BCG, 2) those resulting from the intravenous injection of a large amount of old tuberculin, 3) necropsy studies of anaphylactic shock with ovalbumin or sheep red cells, and 4) histological investigation at short intervals of the lesions in BCG-inoculated rabbits intravenously injected with a residue of tubercle bacilli (RHA), in which no lipoid fraction was detectable. These led to the following results:

1) Severe allergic, exudative change was observed in the lungs of the animals superinfected with BCG. In the reaction a monocytic character was found markedly. Similar findings were noted in the spleen and the liver.

2) The viscera of the animals injected with old tuberculin showed a response almost identical to that of the superinfected animals.
3) In the lungs of the animals which underwent anaphylaxis exudative reaction was restricted to the narrow range around the small blood vessels. However, in those of survived animals the lesions were greater and severer. Thus the findings in anaphylaxis were found to be close in its character to those in delayed shock with the lapse of time.

4) The cell residues (RHA) caused an intense exudative reaction in BCG-inoculated animals. In this reaction nonspecific characteristics were detected somewhat strongly, however, the fundamental change was found to be of allergic origin.

5) From the data thus obtained causal genesis of delayed type reaction, especially of delayed shock was discussed.

An Electrophoretical Study on the Changes by Heating of the Serum Protein Fractions

A. HAGIWARA, T. KOBAYASHI and H. KOSUGI

In order to study the influence of heating on the changes in quantity and electrophoretic mobility of the serum protein fractions, a rabbit serum was heated at 56°C for 5, 10, 20, or 30 minutes. The sera thus treated were studied electrophoretically on a filter paper. The experiment led to the following results:

1) No quantitative change in the total protein concentration was observed in all the sera heated at 56°C.

2) The quantity of albumin increased and that of globulin decreased until 10 minutes. However, from 20 minutes this relation between albumin and globulin became quite opposite. Marked decrease in the quantity of albumin was noted in the sera heated for 30 minutes.

3) Heating caused a decrease in the mobility of all the protein fractions, especially of albumin.