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Epidemiological Study of Tuberculosis by the Phosphatide Hemagglutination Reaction

Kei MASAMUNE

The sensitizing antigens participating in the Middlebrook-Dubos hemagglutination reaction are generally considered to be of polysaccharine nature. Recently, Takahashi and Ono has reported that phosphatides isolated from the tubercle bacillus can also sensitize sheep erythrocytes to hemagglutination in the presence of tuberculous sera both of human and animal origin, and that the antigen-antibody system participating in the phosphatide hemagglutination reaction is independent from that participating in the polysaccharide or protein hemagglutination. This has led the present author to search for the epidemiological significance of the newly established phosphatide hemagglutination reaction.

Forty-six healthy persons who were negative to a 100-fold dilution of Old tuberculin and not vaccinated with BCG, 32 infants, 532 school children, 60 adults, 65 tuberculous infants and 99 tuberculous patients were the objects of the present study. In these subjects both the phosphatide and polysaccharide hemagglutination reaction were tested for comparison. The results obtained are as follows:

1) The hemagglutination titers by the phosphatide antigens were generally lower than those by the polysaccharide ones. The titers of sera of the healthy persons by the polysaccharide antigens formed nearly a normal distribution curve, while those by the phosphatide antigens a logarithmic one. This suggests that the antibodies participating in the polysaccharide hemagglutination are independent of those participating in the phosphatide hemagglutination.

2) Average hemagglutination titers of sera from the healthy persons by the both antigens, phosphatide as against polysaccharide, were as follows: in tuberculin negative individuals, 1.0 : 1.5, in infants, 1.0 : 2.1, in school children in the 1st to 3rd grade, 1.7 : 2.9, in school children in the 4th to 6th grade, 1.7 : 3.5, in junior high pupils, 1.7 : 3.3, in adults, 1.1 : 2.2. No correlation was recognized between the induration sizes due to the tuberculin skin-test and the degrees of the hemagglutination titers.

3) To take as positive reactions above 2 plus, the positive hemagglutination rates (percentages) of the healthy persons, phosphatide as against polysaccharide, were as follows: in tuberculin nonreactors, 4.3 : 3.7, in infants 3.0 : 5.0, in school children, 52.3 : 79.5, in adults with no history of tuberculosis, 11.0 : 66.6, in healthy adults with anamnesis for tuberculosis, 5.0 : 71.4. The high positive rate in the school children seems to be related to BCG vaccination.

4) In tuberculous infants, the average hemagglutination titers was the highest in those with mixed type tuberculosis. The average titers according to the types of the disease, phosphatide as against polysaccharide, were as follows: in mild cases, 1.2 : 5.6, in moderate cases, 2.2 : 5.6, in serious cases, 73.5 : 97.0. The hemagglutination titer was especially high in serious cases. In these cases the maximum titer was 79.4 and the minimum 67.6 for phosphatide while the maximum was 100.10 and the minimum 93.90 for polysaccharide. The titers for polysaccharide were higher in older children. For phosphatide there was observed no significant difference between the older children and the younger ones. The rates of positivity,

phosphatide as against polysaccharide, were as follows: in mild cases, 12.5 : 89.5, in moderate cases, 38.2 : 83.4, in serious cases, 100 : 100. Thus, the phosphatide hemagglutination reaction reflected well the seriousness of the disease, while the polysaccharide reaction did not.

5) In adult tuberculous patients, the hemagglutination titer was high in patients with infiltration and mixed type tuberculosis. The titers according to the types of the disease, phosphatide as against polysaccharide, were as follows: in mild cases, 1.1 : 3.5, in moderate cases, 4.6 : 3.3, in serious cases, 76.81 : 98.7. The titers were especially high in serious cases. In these cases the maximum titer for phosphatide was 79.33 and the minimum titer for polysaccharide was 96.10. The positive rates (percentage), phosphatide as against polysaccharide, were as follows: in mild cases, 9.5 : 78.8, in moderate cases, 69.6 : 87.6, in serious cases 100 : 100. As in the tuberculous infants, the titers by the phosphatide antigens reflected well the importance of the disease, while those by the polysaccharide antigens did not. These results suggest that the hemagglutination reaction by the phosphatides of the tubercle bacillus is considerably valuable for the objective evaluation of the importance of tuberculosis disease.

In vitro Studies on the Antituberculous Activity and Combined Effect of Sulfonamides

Shun TANIWAKI

Using sensitive, SM-resistant and INAH-resistant strains of tubercle bacilli, the antituberculous activity and combined effect with SM and INAH were studied *in vitro* of 7 different kinds of sulfonamides, homosulfamin, sulfathiazole, sulfadiazine, sulfanilylxylamide, sulfisoxazole, sulfisomidine and sulfamethoxyipyridazine. The experiments led to the following results:

1) In vitro antituberculous activity.

Against all the strains, sensitive and resistant, sulfathiazole and sulfisoxazole showed a complete inhibition at 40 μ /ml, sulfisomidine at 50 μ /ml, sulfadiazine and sulfamethoxyipyridazine at 70 μ /ml. Sulfanilylxylamide showed only an incomplete inhibition at 70 μ /ml and homosulfamin no inhibition.

2) Combined effect with SM and INAH

All the sulfonamides tested exhibited no combined effect with SM. When combined with INAH, the sulfonamides except homosulfamin and sulfanilylxylamide showed clearly an additive action against sensitive and SM-resistant strains, but not against INAH-resistant strains.

Sur la déhydrogénation endogène du bacille tuberculeux

Teiko YAMASHITA et Koji SATO

1° Chez les bacilles déséchés par acetone provenant d'une souche de mycobactéries aviaires, nous avons trouvé une activité déhydrogénasique endogène. Nous en avons pu extraire la solution enzymatique et son substrat.

2° Il nous semble que le substrat est un mélange ou un composé de sucres.

3° Les extraits bactériens ont été concentrés par précipitation fractionnée au $\text{SO}_4(\text{NH}_4)_2$, et les fractions comprises entre 0,5 et 1,0 de saturation se sont trouvées données de cette activité enzymatique.

Studies on the Antigen-Antibody Reaction in Agar

II. Examination of Ouchterlony's agar diffusion technique by the use of egg-white, egg albumin and bovine serum albumin

Eiichiro SUMIKAWA and Koji SATO

1) In the agar diffusion precipitin reaction by Ouchterlony's technique, the appearance and the localization of precipitate lines are determined by the antigen/antibody ratio.

- a) With increasing relative concentrations of antigen the precipitate lines are formed closer to the antibody reservoir and with increasing relative concentrations of antibody closer to the antigen reservoir.
- b) In excess of antigen, the precipitate lines formed move slowly with time towards the antibody reservoir, since the precipitates dissolve in the diffusing antigen in excess and are re-formed closer to the antibody reservoir. In excess of antibody, the precipitate lines move slowly towards the antigen reservoir by the same mechanism.
- c) If the initial concentrations of antigen and antibody are in an optimal ratio, the precipitate lines once formed move to neither of the directions, staying always at the same position.
- d) No precipitate band is formed, when either antigen or antibody is too in excess.

2) By the agar diffusion technique, egg albumin and anti-egg albumin rabbit sera used in this experiment give rise to 2 precipitate lines, egg-white and its antisera 4, and bovine serum albumin and its antisera 2.

3) In Ouchterlony's agar diffusion precipitin reaction, two kinds of procedures for absorption of antibody are available. The first one is achieved by direct introduction of antigen (or antibody) into the agar before the precipitin test is started, that is, all the reservoirs of agar plate are filled for 3 consecutive days with antigen (or antibody), which is then allowed to diffuse completely into the agar in order to keep the medium in excess of antigen (or antibody). The second one is the ordinary absorption technique for antibody in the test tube and the antisera obtained by the specific absorption are used for the reactant in Ouchterlony's technique. These two absorption procedures lead to the same result in agar diffusion, but they have their own advantages and disadvantages.

In the second procedure for absorption of antibody, the quantity of antigen to be used for absorption must be so strictly calculated that it may be in the optimal proportion to the quantity of antibody in order to make the absorption complete. In this connection, the first procedure is technically more simple, because it does not demand to calculate the quantity of antigen, but it has a disadvantage of taking a very long time for absorption. Therefore, it is necessary to choose between the two procedures according to the number of antigenic components involved or experimental purpose etc.

Studies on the Metabolism of Tubercle Bacilli

III. On the amino acid metabolism

Koji SATO and Eiichiro SUMIKAWA

Twelve amino acids were examined for their effects on the respiration of *M. avium* (strain Takeo), *M. phlei*, *M. tuberculosis* var. *hominis* (strain H37Rv and H37Ra) and BCG, with special reference to

the effects of l-cysteine on the oxygen uptake and dehydrogenase activity of these strains as well as the inhibition of its activity by KCN. In parallel, the effect of l-cysteine on the growth of *M. avium* and the effect of Sauton's medium plus l-cysteine or culture filtrate of Takeo strain plus l-cysteine on the respiration of *M. avium* was examined. The results obtained were as follows:

- 1) *M. avium* and *M. phlei* actively utilized more kinds of amino acids for respiration than did the other 3 strains.
- 2) L-cysteine showed a remarkable stimulatory effect on the oxygen uptake of all the strains of mycobacteria tested (optimal pH: 7.0) but this effect was considerably inhibited by the addition of KCN.
- 3) Next to l-cysteine, l-glutamic acid showed a stimulatory effect on the oxygen uptake of these strains.
- 4) Glycine, dl- α -alanine, l-aspartic acid and lysine were oxidized by *M. phlei* and *M. avium*, and dl-valine and l-histidine by *M. phlei* respectively, but these amino acids were not or hardly oxidized by the other strains of mycobacteria tested.
- 5) Dl-serine, dl- β -phenylalanine and tryptophan showed almost no effect on all the strains tested.
- 6) Of the five strains of mycobacteria tested, *M. phlei* and *M. avium* exhibited the strongest endogenous dehydrogenase activity, followed by BCG, while both H37Rv and H37Ra were weaker than the others. However, when l-cysteine was added as substrate, it stimulated most remarkably the dehydrogenase activity of H37Rv and H37Ra.
- 7) L-cysteine was always inhibitory for the growth of *M. avium* except when a very small amount of this substance, 0.0000025 M, was added in Sauton's medium containing no amino acid. The fact that l-cysteine is stimulative for respiration in spite of being inhibitory for growth is considered to be of great interest.
- 8) Oxygen uptake of mycobacteria by the addition of l-cysteine plus Sauton's medium as substrates appeared to be somewhat different from that of mycobacteria by the addition of l-cysteine plus phosphate buffer. Mycobacteria of younger cultures cultivated in alkaline medium were not so much stimulated of their oxygen uptake by the addition of l-cysteine than was expected.

Stress and the Urine Quotient

—Studies for Evaluation of the New Urine Quotient O/K₃ Method as a Means for Investigating Industrial Fatigue—

HIROSHI YOKOYAMA

Studies were made for evaluation of the urine quotient O/K₃, a living body vitality criterion which has recently been applied to the quantitative and qualitative determination of supplementary fluids in the field of surgery. The purpose of the present studies was to determine whether the method based on the urine quotient O/K₃ is suitable as a fatigue criterion for industrial fatigue. For this purpose reevaluations were made, with lumber jack fatigue as the object.

Four cases including one case of hypertension (subject I. E.), were investigated. Labor conditions, under which the subjects were studied, were *Whip saw felling* (R. M. R.: 5-7) and *Chain saw felling* (R. M. R.: 3-5). The amount of timber felled was set at 1² × 36 m³.

Urine samples were collected early in the morning (1st urine), after morning work (2nd urine), afternoon work (3rd urine), upon return to *Bunk house* (4th urine). A total of 108 samples were collected.

1) No correlation was seen between O/K₃ values and values of the 13 urine biological substances (reactions) including urine pH, measured concomitantly.

2) Maximal values in O/K₃ were seen in the urine samples taken in the morning (2nd urine), when the largest amount of *timber* was felled.

3) O/K₃ values in subject I. E. were highest throughout all the labor conditions as compared with the other 3 subjects.

4) The values of subject I. E. were higher in mechanical labor (Chain saw felling) than those in Whip saw felling.

From these results, it may be concluded that the present urine quotient O/K₃ is highly effective as an industrial fatigue criterion.

On the Qualitative and Quantitative Determination of Supplementary Fluids for Thoracic Surgery

—5 per cent Glucose Solution—

Yoshijiro KITAMURA

With the intent of clarifying the significance and value of glucose solution as a supplementary fluid in the field of lung surgery, the author selected 15 tuberculous patients who had undergone thorocoplasty. Studies were made of 15 biological substances (reactions) in blood and urine centered around the urine quotient O/K₄, a vitality criterion of the living body. The results were as follows.

In this case, the condition of transfusion for 5 per cent glucose solution was as follows: 1500 cc on the day of and during operation, 1000 cc on the 1st post-operative day, 500 cc on the following day, totaling up to 3000 cc.

Further, blood was transfused to cover approximately the amounts of blood lost (300~500 cc), the closed circulatory anesthesia apparatus being not used during operation.

Results: Pre- and post-operative fluctuations in the urine quotient were as follows.

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|-----------------------------|-------------|
| 4-3 pre-op. days | 49.8±4.50 |
| 2-1 pre-op. days | 60.5±9.34 |
| 0 day immediately after op. | 178.8±20.82 |
| 1st day after op. | 83.7±10.57 |
| 2nd day after op. | 67.7±6.05 |
| 3rd day after op. | 54.7±4.00 |
| 4th day after op. | 52.7±3.36 |
| 6th day after op. | 66.4±13.21 |
| 8th day after op. | 51.1±4.95 |
| 10th day after op. | 80.0±25.66 |

It is noted that, as compared with the corresponding values as obtained by Takeuchi by using an un-mixed transfusion of physiological saline solution, the present values are smaller: it indicates that the present solution is a comparatively excellent transfusion solution.

However, in spite of the above even in the present solution, there is a definite side reactions coming from excessive transfusion.

This is seen in the following:

- i) Post operative first half—from 0 day to 4 days after operation.

In half of the subjects, an abnormal decrease in protein metabolism coming from over-administration of the present solution was present.

ii) Post operative latter half—from 8 to 10 days after operation.

In half of the subjects an abnormal rise in adrenal function was present.

On the Qualitative and Quantitative Determination of Supplementary Fluids for Thoracic Surgery

— a Mixture of 5 per cent Glucose Solution
and Physiological Saline Solution (2:1)—

Yoshiharu ODA

With the intent of determining the feasibility of a 2 : 1 mixture of 5 per cent glucose and physiological saline solution (G-P mixture) as a transfusion solution for thorocoplasty, the author selected 13 male tuberculous patients. Studies were centered on the measurement of the urine quotient (O/K_4) and measurements were made on various biological substances in urine and blood.

Condition of transfusion for G-P mixture: 1500 cc on the day of and during operation, 1000 cc on the 1st post-operative day, 500 cc on the following day, totaling up to 3000 cc.

Results: Pre- and post-operative fluctuations in the urine quotient were as follows.

| | |
|-----------------------------|-------------|
| 4-3 pre-op. days | 44.9±2.86 |
| 2-1 pre-op. days | 55.0±4.03 |
| 0 day immediately after op. | 114.3±24.14 |
| 1st day after op. | 72.9±4.03 |
| 2nd day after op. | 62.3±5.31 |
| 3rd day after op. | 60.4±2.99 |
| 4th day after op. | 57.5±5.55 |
| 5th day after op. | 65.4±7.70 |
| 6th day after op. | 67.1±8.67 |
| 8th day after op. | 53.3±6.05 |
| 10th day after op. | 51.9±3.85 |

It is noted that, as compared with the corresponding values as obtained by Takeuchi and/or Kitamura by using unmixed transfusions of physiological saline solution and/or glucose solution, the present values are much smaller: it indicates that G-P mixture is a comparatively excellent transfusion solution.

However, in spite of the above even in the present mixture as a transfusion solution, there are definite side reactions coming from excessive physiological saline.

This is seen in the following:

i) Post operative first half—from 0 day to 4 days after operation.

In half the subjects, difficulty in urine elimination, abnormal systemic reactions coming from transition of sodium, especially chlorine into tissue were present.

ii) Post operative latter half—from 8 to 10 days after operation.

In half the subjects, a secondary dehydrative reaction coming from a prolapse of inorganic salt accompanied by a rise in the systemic catabolic reactions was present.

From the above result, it may be concluded that, while the present mixture solution has the above side effects due to a comparatively excessive amount of sodium in the mixture, it is decidedly favorable as

a supplementary fluid in the field of thoracoplastic surgery, as compared with the single use of glucose solution or physiological saline solution.

Immunological Study of Sera of Tuberculous Animals

V. Part II. Localization of Anti-Tuberculin Antibodies in Tuberculous Sera

AKIO HAGIWARA

Using the starch electrophoretic technique the localization of various anti-tuberculin antibodies in sera of tuberculous rabbits was studied. The serum protein fractions were identified by paper electrophoresis.

The serum precipitating antibody to tuberculo-protein was found in the slow moving γ -globulin fraction, while the skin-sensitizing antibody in the fast moving γ -globulin fraction. The sensitivity thus transferred in rabbits with concentrated eluates from the starch block containing the γ -globulin was of delayed type. However, the hemagglutinating antibody (Middlebrook & Dubos) and the hemolytic antibody (Middlebrook, 1950) to tuberculin polysaccharide and the complement-fixing antibody to old tuberculin were found in the whole γ -globulin fraction. Furthermore, the titers of these three kinds of antibodies in each eluate from the starch block containing the γ -globulin region were related to the concentration of this protein fraction.

Experimental Studies on Tuberculin Type Hypersensitivity Using the Suspension Culture Technic

3. The Role of the Cellular and Humoral Factors in Ovalbumin Cytolysis

MIKIO ITO

Using the suspension culture technic as described in a previous paper, splenic cells of normal rabbits and those sensitized to crystalline ovalbumin were cultured in the presence of ovalbumin in the culture media containing either normal or immune serum.

In the absence of ovalbumin, the proliferation ratio of normal and sensitized animals was equal to that observed when cultured either in normal or immune serum.

In the presence of normal serum, the proliferation of normal cells was not inhibited by ovalbumin, while, in the presence of immune serum, the proliferation took place only partially. Similarly, the proliferation of sensitized cells was not markedly inhibited by ovalbumin in the presence of normal serum, while, in the presence of immune serum it was considerably inhibited.

To compare the data thus obtained with those reported in a previous paper, it is clear that both cellular and serum factor are concerned in specific cytolysis by ovalbumin, as well as by tuberculin. However, in Arthus type hypersensitivity, the serum factor plays an important role, while, in tuberculin type one, the cellular factor does so.

Studies on the Antigenicity and Immunogenicity of the Protein and Polysaccharide Fractions of Tuberculin

Toyaji KOBAYASHI

Protein and polysaccharide (a fraction precipitated at 40 per cent MeOH) were fractionated from non-heated culture filtrates of *Myc. tuberculosis*. Rabbits were injected with these fractions with adjuvant. Thirty days after the injection the animals and normal control rabbits were challenged with virulent tubercle bacilli. At different intervals they were sacrificed and the visceral organs were studied histologically.

Injection of the protein fraction provoked a strong skin sensitivity and increased the serum precipitin titer to the antigen in rabbits, both being decreased following challenge. Similarly, injection of the polysaccharide fraction increased considerably the hemagglutinin titer (Middlebrook-Dubos) to a polysaccharide fraction (precipitated at 95 per cent MeOH). The serum γ -globulin, which showed a gradual increase in the quantity in the course of the sensitization, decreased strikingly following challenge.

Three and seven days after challenge the lungs of the sensitized animals showed an intense exudative inflammation. However, 30 days after challenge tuberculous changes in the animals were almost equal in the severity to those of the non-sensitized control animals.