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Study on the Hemagglutination Reaction by the Phosphatide of the Tubercle Bacillus

Examinations on the Conditions of the Procedure

Katsuo Ono

For the purpose of determining the best conditions for the procedure of the phosphatide hemagglutination reaction, experiments were performed using a stock solution of methanol containing purified tubercle-phosphatide at a rate of 2.0 mg per ml. In parallel to these experiments, comparison was made, using the same phosphatide antigens, between the complement fixation reaction, the precipitation reaction, the conditioned hemolysis reaction and the hemagglutination reaction. The following were the results:

1) As for the method of preparing sensitizing antigens, the method in which the stock solution is added drop by drop into saline or buffered saline under constant agitation by means of a magnetic stirrer or by hand was found to be most favorable. The antigens thus obtained were stable for a relatively long period of time at room temperature as well as in a refrigerator. Sheep erythrocytes sensitized with these antigens give the highest antibody-titers.

2) The optimum pH values for the reaction lie between pH 7.0 and 8.0.

3) For sensitizing erythrocytes as well as for performing the hemagglutinations, the condition of 37°C 30 minutes was most favorable.

4) The addition of suitable quantities of guinea-pig complement to the hemagglutinating media led to the hemolysis reaction. The hemolysis titers increased to a certain extent with the increase in quantities of the complement added. The hemolysis titers were 1/2 to 4 times as high as the hemagglutination titers.

5) The antibody titers given by the complement fixation reaction and the precipitation reaction were much lower than those of the hemagglutination.

6) The addition of ovolecithin to the phosphatide antigens did not increase the antibody titers to be given by the latter alone.

Study on the Clinical Significance of the Population Structure and Catalase Activity of Isoniazid-resistant Tubercle Bacilli

Tadazumi Onodera, Tadashi Kuwajima, Hajime Fukae, Isamu Urushihara, Hideo Ono and Eiichi Sakai

Population structure and catalase activity were systematically studied for a period of 7 months on strains of tubercle bacilli isolated from sputa of 97 patients with pulmonary tuberculosis, in comparison to the clinical status of the patients. The catalase activity was measured according to the bubbling
method of Middlebrook, using an equivalent mixture of 30 percent liquor hydrogenii peroxydati and 0.1 percent solution of Tween-80. The study led to the following results:

1) No parallelism was found between the degree of isoniazid-resistance of the tubercle bacilli isolated and the quantities of isoniazid administered. It was not seldom that high resistant strains were isolated from patients who received relatively small amounts of the drug and vice versa.

2) The intensity of catalase activity of the strains were not related to the amounts of the drug administered.

3) Catalase was always positive for sensitive strains. It diminished gradually with the increase in drug-resistance of the bacilli: the diminution being 31 percent for 0.1γ resistant bacilli, 64 percent for 1γ-resistant bacilli and 81 percent for 10γ-resistant bacilli.

4) For the bacilli of low resistance of 0.1 and 1γ, no definite relationship was observed between the intensity of their catalase activity and their population structure. On the contrary, for the bacilli of high resistance, a tendency was noticed that their population structure becomes homogeneous.

5) The bacilli isolated from patients resistant to the drug showed almost all an uneven population structure. Only a few of them showed 100 percent resistance. The population structure of the drug-resistance showed almost always a definite tendency for each individual.

6) In patients having 10γ-resistance, those who showed a homogeneous population structure for their bacilli followed a relatively favourable progress, as compared with those showing an uneven population structure. However, the intensity of catalase activity of the bacilli isolated was of no use in presuming the prognosis of the patients.

Clinical Study on the Blood Level of Isoniazid in Patients with Pulmonary Tuberculosis

(II) Sputum-negative Cases following Isoniazid-treatment

Tadazumi ONODERA, Hideo ONO, Tadashi KUWAJIMA, Hajime FUKAE and Isamu URUSHIBARA

The blood level of isoniazid was measured, according to Ogawa's diffusion method, in patients with pulmonary tuberculosis who became early sputum-negative following isoniazid-treatment. This was done for the purpose of knowing the relationships, if any, between the blood level of the drug and its efficacy. The results were as follows:

1) The blood level of isoniazid showed great individual differences.

2) When administered in combination with PAS, isoniazid reached a blood level higher than when administered alone, two hours after administration, but, 6 hours after administration, no retardation was noticed in its inactiveness.

3) The blood level was higher in patients who became sputum-negative during 1 to 4 months after the beginning of isoniazid-treatment, than in those who did so after more than 5 months.

4) The blood level of isoniazid was found to be related neither to the types of the disease nor to its clinical efficacy.
On the Qualitative and Quantitative Determination of Supplementary Fluids in Tb Surgery
—Physiological Saline Solution or Ringer's Solution—

Hide TAKEUCHI

In recent years, in the field of surgery the advancement of chemotherapeutics and surgical technics have enlarged the application of surgery to a great extent. While this is a matter to be met with approval, it seems a pity that both in blood transfusion and in infusion solutions no definite quantitative or qualitative conditions have been set forth.

As part of a series of studies, with special regards to the determination of the conditions in question, the author has conducted a series of investigations with the focus of attention on urine quotients, in order to determine the type of Ringer's solution or physiological saline solution in the above mentioned lung operation.

Conditions in the present studies. The male Tb patients under investigation were divided into the 2 following groups. Both the physiological saline solution group (P.S.: 15 patients) and Ringer's solution group (R: 24 patients) were subjected to thorocoplasty (closed circulatory anesthesia not used). Infusions were made in both groups; during and immediately after operation (0 days) 1500 cc, on the first day after 1000 cc and on the second day after 500 cc, a total of 3000 cc. Blood transfusion in approximately equal amounts to blood lost (300~500 cc) were also administered. One day urine samples were taken from 2~4 days prior to operation and for 10 days after (2 weeks). Blood samples (blood serum) were taken in the early morning on the day of operation (prior to), the day after, the 3rd, the 6th, the 8th and the 10th day after operation, a total of 5 days.

In order to determine the type of infusion solution, a new urine quotient (O/K.) was used. The normal value of the quotient in question stands at approx. 30 which rises with the lowering of the vitality of the living body and with the lowering of adaptation; the value reaches its maximum immediately prior to death. As the next step the following substances were measured in blood serum with the aim of identifying the cause of lowering of vitality following operation in both groups, with the metabolism of various substances; Na, K, Cl, Na/K, Na/Cl and iodate value. On the other hand the following measurements were made on; urine volume, pH, Na, K, Cl, N and O/N.

The urine quotient values in both the P.S. group and R group were as shown below. On the underlined days in the P.S. noteworthy high values were seen. Especially, in the P.S. group immediately after operation, it was noted that an almost maximum value was seen. (Fig. 1, 2)

<table>
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<tr>
<th>Days after Operation</th>
<th>P.S. Group</th>
<th>R Group</th>
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<tr>
<td>1 day prior to op.</td>
<td>71.8±9.78</td>
<td>46.7±5.78</td>
</tr>
<tr>
<td>Immediately after op.</td>
<td>395.1±68.8</td>
<td>136.6±15.56</td>
</tr>
<tr>
<td>First day after</td>
<td>149.9±5.73</td>
<td>67.4±9.95</td>
</tr>
<tr>
<td>Second</td>
<td>100.8±32.9</td>
<td>43.0±2.27</td>
</tr>
<tr>
<td>Third</td>
<td>86.0±25.4</td>
<td>44.5±6.66</td>
</tr>
<tr>
<td>Fourth</td>
<td>50.1±4.90</td>
<td>45.2±7.57</td>
</tr>
<tr>
<td>Fifth</td>
<td>53.0±5.74</td>
<td>52.4±10.56</td>
</tr>
<tr>
<td>Sixth</td>
<td>66.4±7.23</td>
<td>37.5±1.85</td>
</tr>
<tr>
<td>Eighth</td>
<td>61.9±4.21</td>
<td>34.9±1.87</td>
</tr>
<tr>
<td>Tenth</td>
<td>69.3±8.80</td>
<td>39.6±7.88</td>
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The following facts worthy of being noted are listed as follows:

...
(A) Prior to operation: Higher values in O/K4 were seen in the P.S. group as compared with the R group, whereas, a rise in O/K4 value indicates a lowering in the vitality of the living body, the difference as seen in both groups largely depends on the environment to which the living body is exposed. It is conjectured that the higher values in the P.S. group are due to stress resulting from mental factors such as anxiety and fear of the operation etc. Besides the above, in the P.S. group as compared against the R group, noteworthy differences were seen in urine pH, urine O/N in which high values were seen together with low values in chloride discharge.

(B) Immediately after operation (0 days): Whereas in the P.S. group urine quotients showed an almost maximum value, in this case as compared with the R group the lowering of urine pH is not marked. From this, it is conjectured that a delay in the onset of normal living body reaction to operative stress was brought about in the P.S. group. Especially, in the 7 cases (half of total) in the P.S. group in which the O/K4 values exceeded 400 not only was there a suggestion of difficulty in urine discharge but also a highly abnormal (stress) in various metabolic activities (fig. 5). In general it may be said that there is a suggestion of delayed abnormal hyperfunction and/or a suggestion of an abnormal hyperfunction of the adrenal gland and/or further a suggestion of dehydration or hydremia which in turn suggests, organ edema during or immediately after operation resulting from P.S. administration (table 4, 5).

(C) 1–3 days after operation: The O/K4 values in the P.S. group as compared with the R group was high. It was noted that in all cases or half of the number of the P.S. group the pH recovery to normal was delayed. Serum potassium showed low values and urine nitrogen showed low values. Urine O/N showed high values while the serum iodate number showed low values. Thus, in regards to P.S. solution as an infusion solution, it was determined that this solution has a strong inhibitory effect on the hyperfunction (dissimilation) of protein metabolism which as a normal living body reaction counteracts post-operative stress (table 4, 6).

(D) 5~10 days after operation: In regards to the O/K4 value in the P.S. group, especially 6~10 days after operation as compared with R group, higher values were seen, indicating a remaining lowering of vitality as an aftermath of the operation. In this case, in the P.S. group a high value in urine discharge together with a low value in urine chloride concentration was seen. Especially in half of the P.S. group a rise in urine and blood potassium together with a large dispersion in various organic substances (reaction) was noted. Thus, in special regards to the P.S. solution group, in the latter part of the post-operative course, a definite weakness is seen in metabolic function.

Thus, it may be concluded that P.S. solution as an infusion solution as compared with that of R solution may at times bring about a state of shock in the living body, may inhibit the counteraction of the normal living bodies reaction to post-operative stress, combining symptoms of low potassium in blood, and may cause a delay in post-operative recovery. Hence, it is concluded that at least within the field of lung surgery the administration of P.S. solution during or after operation should be strictly avoided.

On the Fluctuations of Sodium, Sugar, Protein, Acetone Bodies and Urobilinogen and Volume in Urine in the Presence of Various Transfusion Solutions in Lung Resection

Osamu Nishikaze, Yoshihara Oda, Hiroshi Nishimura, Tomio Gocho, and Hiroshi Yokoyama

Three groups were set up according to transfusion conditions in Lung Resection.

1) In the first group, in addition to the transfusion of bank blood amounting to the same blood...
lost, a mixture of 5% glucose and physiological saline solution is administered at the rate of 1000 cc during operation and 500 cc after operation, plus 500 cc in the morning and afternoon of the second day after operation, plus 500 cc on the 3rd day.

2) In the second group, in addition to the transfusion of bank blood amounting to the same blood lost, a mixture of 5% glucose 2000 cc and Ringer's 1000 cc (2:1) is administered in the same pattern as in 1).

3) In the 3rd group, no transfusion solutions were used. Blood transfusion alone, using stored blood amounting to equal volumes of the blood lost during operation were administered.

In the 3 groups as shown above, from 2 days prior to operation to 5 days after, 24 hour urine was collected every day, and from the 7th day after to the 11th day, 24 hour urine was collected every other day. Measurements were made on urine volume, sodium, sugar, protein, acetone bodies and urobilinogen.

The results were as follows:

1) When the degree of diuresis by urine volume was compared in the 3 groups, it was noted that in the 2nd group, prior to and after operation the degree was favorable while in the 1st group in the latter half oliguria was present and in the 3rd group oliguria was present post-operatively.

2) As regards the fluctuations in urine sodium, the urine volume in the 1st and 2nd group were approximately in parallel with each other and in the 3rd group in the latter half the parallelism ceased, suggesting that sodium had dried up.

3) Concerning the appearance of sugar in urine, a positive appearance in a slight degree was present in the 1st and 2nd group. Yet, when its mechanism is taken into consideration, it seemed unnecessary to worry about this appearance since such an appearance may cause no hinderance. In the 3rd group, with the exception of one case on a single occasion when urine sugar showed positive, all cases showed negative.

4) In regards to the appearance of urine protein the 3rd group showed negative values in all cases and in the 1st and 2nd group very mild positive values were seen. This suggests a slight stimulation of the Kidney.

5) With reference to urine acetone body appearance the 3rd group, as compared against the 1st and 2nd, showed a higher positive degree with a longer duration. This may be due to the fact that supplementary fluids have an advantageous influence on lipide metabolism and general metabolism.

6) In regards to the appearance of urobilinogen in urine, the 3rd group, as compared with the 1st and 2nd, shows a high degree of appearance post-operatively for several days and disappears on or after the 7th day. Contrary to the above, the 1st and 2nd group (possibly for the protection of the liver) show lower positive values as compared to the 3rd group. However, as seen in the 2nd group, a continuance of positive values is still seen even after the 7th day. Now could this be due to the influence of potassium on the heart muscle and when this is considered in connection with the appearance of urine protein, it seems that it suggests indications of the Liver-Kidney syndrome. In any event, in regards to the volume of Ringer's solution to be used, close to ideal results may be obtained by a re-evaluation of the amount to be used.
On the Difference in Oral and Armpit Body Temperature Readings in Tuberculous Patients

Osamu Nishikaze, Yoshiharu Oda, Takashi Oguri, and Hiroshi Yokoyama

The authors conducted a study on 49 male tuberculous patients. Oral and armpit temperature readings were taken for 30 days, 4 times a day (total 120 times). The following results were obtained.

I) Both temperature readings were made at 06.00, 09.30, 14.00 and 21.00 hours respectively. It was noted that in both temperatures the values were higher in the afternoon.

II) i) The above subjects were divided into the following 2 groups and studied.
A group: This group consists of subjects showing lower values (−), 9 times or under, in oral as compared against armpit temperature, out of 120 readings.
B group: This group consists of subjects showing lower values (−) 10 times or over as in the above.
It was noted that the appearance of (−) values in A group 3–5 times out of 120 readings, whereas, that of B group was 20–50.

ii) In regards to the above temperature difference, from a point of view of time, the rate of appearance of (−) Value was higher in the morning hours especially in the early morning as compared against the afternoon hours.

iii) In regards to the causation of the difference in temperature, no difference was seen in oral temperature between the two groups, in armpit temperature a definite difference was seen with significantly high values in B group.

iv) When age segments were made in the above two groups, it was noted that B group consists largely of lower age subjects (20–29), and in blood pressure segments a larger number with higher blood pressure were found in A as compared with B.

v) When Chest X-ray (W.H.O.) segments were made, in advanced stages were seen from a chest diagnostic point of view.

vi) Whereas, when the following segments (ie comparative body weight, blood pressure, tuberculin, blood sedimentation rate, and expectoration per day) were made, no significant difference was seen between A and B.

Experimental Studies on the Tuberculin Type Hypersensitivity Using the Suspension Culture Technic

2. The Role of the Cellular and Humoral Factors in Tuberculin Cytolysis

Mikio Ito

In order to verify the role of cellular and humoral factors in specific cytolysis by the antigen-antibody reaction, using the suspension culture technic described in the preceding paper, splenic cells of normal and BCG-vaccinated rabbits were cultured in the presence of old tuberculin in the culture media containing either normal or immune serum.

In the absence of tuberculin, the proliferation ratio of the cells of normal and vaccinated animals was equal when cultured either in normal or immune serum. In normal serum, the proliferation of
normal cells was not inhibited by tuberculin, while, in immune serum, partial proliferation took place.

On the other hand, the proliferation of immune cells was conspicuously inhibited by tuberculin both in normal and immune serum.

It is evident from the results that both cellular and serum factors play a certain role in specific cytolysis by tuberculin.

**Influence of Adrenocortical Hormone on the Immunologic Reaction as Observed in Rabbits Challenged with Virulent Tubercle Bacilli**

Tetsuji Hashimoto

Rabbits previously immunized with BCG were challenged intravenously with virulent tubercle bacilli. From one week on before challenge the half of the animals received daily subcutaneous injections of prednisolone until the end of the experimentation. The tuberculin skin reaction and the serum precipitin reaction were tested weekly. The content of the serum protein fractions were measured using paper electrophoresis. The animals were sacrificed 1, 3, 7, 14 and 30 days after challenge. Histological study of the lesions of their viscera was performed.

A marked inhibition of the tuberculin reaction occurred following prednisolone injections. The precipitin titer and the γ-globulin content was low in the sera of the treated animals. An intense exudative reaction as observed in the non-treated animals 1 and 3 days after challenge was almost completely inhibited by prednisolone. The development of tubercles, especially of epithelioid-cell tubercles in the viscera were also retarded by prednisolone. However, it had no influence on the numbers of viable bacilli until 2 weeks after challenge. In spite of the inhibitory effect on the development of initial exudative and productive changes, 30 days after challenge, the lesions of the lungs and the liver were more pronounced in the treated animals than in the non-treated ones.

From the results, prednisolone was found to have an inhibitory action on the antibody production and on the manifestation of the acute allergic reaction in tuberculous rabbits.