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Abstracts of "Tuberculosis Research" Vol. 12, 1959

A Tissue Culture Test for Evaluating the Virulence of Tubercle Bacilli

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In order to evaluate in vitro the virulence of tubercle bacilli, a tissue culture test was devised which exists in culturing splenic cell suspensions of guinea pigs inoculated intravenously with the bacilli to be tested. The grade of virulence was estimated by the increasing ratio of the bacilli that had multiplied in the presence of the live tissue cells.

In order to confirm its availability for virulence testing, the present test was compared with the four conventional virulence tests, using 9 stock strains and 29 wild strains isolated from patients with pulmonary tuberculosis.

The multiplication of bacilli in the tissue culture ran parallel with the grade of virulence of each of the strains tested. The present test correlated well with the guinea pig inoculation test rather than the mouse inoculation test. The neutral red test and the cord test were found unsuitable for measuring the virulence of the tubercle bacillus, since the results obtained from those tests were often found considerably discordant with those of the animal inoculation tests.

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

Shun TANIWAKI

In a previous paper (this journal, vol. 11), the in vitro antituberculous activity and combined effect of 7 different kinds of sulfonamides was reported. Afterwards, similar studies were made with 8 other sulfonamides. In addition, for the purpose of getting information of the mechanism of the antituberculous activity of sulfonamides, some compounds, other than sulfonamides, which contain a thiazole nucleus or a pyridine nucleus were synthesized and tested in vitro of their antituberculous activity.

Putting together all the results from the point of view of chemical composition of the agents, the following conclusions were drawn:

1) The antituberculous activity of sulfonamides is irrespective of SM and INH resistance of the tubercle bacillus.
2) Sulfonamides which possess antituberculous activity are N′-substitutions of the sulfanyl radical.
3) Sulfonamides which possess combined effect with INH are only N′-heterocyclic substitutions of the sulfanyl radical.

4) Sulfonamides so far tested have no combined effect with SM.
5) The sulfanyl radical itself has no antituberculous activity. The antituberculous activity of sulfonamides results from the combination of some compounds (thiazole, pyridine etc.) to N′-position of the sulfanyl radical.

Experimental Evaluation of Various Hemagglutination Tests for Diagnosing Tuberculosis

Tadazumi ONODERA

Using, as the antigens, samples of polysaccharide, protein and phosphatide of the tubercle bacillus, Middlebrook-Dubos' hemagglutination test, Boyden's hemagglutination test and the phosphatide hemagglutination test, which had been first developed in this laboratory, were periodically performed for 12 weeks with sera from rabbits experimentally infected with tubercle bacilli. For comparison of virulence of the bacilli and the mode of infection, cultures of BCG or highly virulent human tubercle bacilli, strain Nakano, were injected into each group of animals subcutaneously or intravenously. In parallel with these experiments, the influence of the treatment by SM and INH on the antibody production was observed.

Anti-polysaccharide antibodies, as revealed by Middlebrook-Dubos' test, appeared very early after infection, followed by anti-protein antibodies as revealed by Boyden's test. These two kinds of antibodies lasted to exist in the sera almost irrespective of the virulence of the strains used and the mode of infection. They were hardly influenced by the treatment with SM and INH. On the contrary, anti-phosphatide antibodies, as revealed by the phosphatide hemagglutination test, were found to reflect well the virulence of the strains and mode of infection: they were proportionally high in the animals infected with the virulent strain and in those infected intravenously. They were also influenced by the treatment with the antituberculous agents, gradually disappearing with healing of the tuberculous lesions.

These results would indicate that, of the three hemagglutination tests, the phosphatide hemagglutination test is the most valuable for diagnosing the activity of tuberculosis in human clinic.

Studies on the Tuberculin Allergy by Tissue Culture

Tatsuo NUMATA

By the use of the replicate tissue culture technique, cells derived from guinea pigs immune or non-immune to tuberculosis were cultured in vitro in the presence of tuberculin, in order to examine the cellular sensitivity to tuberculin. In this study, cells derived from guinea pigs immunized with heat-killed human-type tubercle bacilli (Strain Nakano) were regularly injured to exhibit cytolysis by the addition of old tuberculin (diluted 1:10), whereas cells from normal guinea pigs were never injured, even when they were cultured in the presence of anti-tuberculous plasma. This finding was in contrast to the data given by O'Neill and Favour that the main principle of cellular tuberculin allergy was mediated by factors present in plasma and that the plasma factors were capable of rendering normal cells sensitive to tuberculin.

Occurence of Tuberculin Type Skin Hypersensitivity during the Development of Sensitization to Ovalbumin, with Special Reference to the Immunological Significance of Tuberculin Type Cutaneous Allergy

Satoshi FUJIMOTO

In this study, observations were made of occurrence of tuberculin type cutaneous hypersensitivity during the development of sensitization to ovalbumin in guinea pigs.

1) The tuberculin type of response was observed in the earlier stage of sensitization, being replaced by the ordinary Arthus type of response in the later stage where sensitization became more complete. The tuberculin type and the Arthus type of cutaneous hypersensitivity can thus be different phases of the same immune process. In this regards, it can be said that tuberculin type hypersensitivity is an "immature form" of Arthus type hypersensitivity.

2) Occurrence of tuberculin type cutaneous hypersensitivity was also found to be related to the doses of antigen injected. When guinea pigs were sensitized with larger doses of ovalbumin, typical Arthus type hypersensitivity developed relatively soon after a short duration of the latent period, so that the period of tuberculin type hypersensitivity became shorter. On the contrary, when guinea pigs were sensitized with smaller doses of ovalbumin, the latent period as well as the period of tuberculin type response were prolonged. To sum up, the increase of the sensitizing dose decreased the duration of the tuberculin type of response, whereas the decrease of the dose prolonged this type of response.

3) When the sensitization reached its maximum, a negative phase developed during which time the skin remained non-reactive to smaller test doses of ovalbumin.

4) The significance of the tuberculin type cutaneous allergy in the immune process were discussed from the results mentioned above.

Studies on the Fatty Acid Metabolism of Tubercle Bacilli

Koji SATO

In this paper, the effects of a wide range of concentrations of a number of fatty acids from C_1 (formic acid) to C_{18} (stearic acid) upon the growth and respiration of avian type tubercle bacilli (Strain Takeo) were examined, with special reference to the effects of lauric and oleic acid upon the viability, metabolism and dehydrogenase activity of this strain.

Results obtained were as follows:

1) All fatty acids employed showed in low concentrations more or less stimulative effects on the growth of avian tubercle bacilli. Lactic acid and butylic acid served as best carbon sources for growth, followed by propionic acid, caproic acid and acetic acid, while lauric and oleic acids were proved to have least effects on supporting growth.

2) All fatty acids employed showed in high concentrations inhibitory effects on the growth of avian tubercle bacilli. These effects were most obvious with lauric and oleic acids, being least with lactic acid.

Fatty acids from C_1 to C_{12} showed increasing inhibitory effects with the increase in the number of their carbon molecules, but higher fatty acids from C_{14} to C_{18} showed, on the contrary, decreasing inhibitory

effects with the increase of their carbon molecules.

3) All fatty acids employed stimulated the oxygen uptake of avian type tubercle bacilli in an adequate range of concentrations. Of the fatty acids tested, lactic acid showed a most remarkable effect on the oxygen uptake of the bacilli, and caprylic and caproic were in this effect next to lactic acid.

No relationship was noticed between the concentrations of fatty acids optimal for growth of bacilli and those optimal for oxygen uptake. In other words, fatty acids which stimulated oxygen uptake to a greater extent did not necessarily support growth best, and vice versa.

4) The viable cells in the suspensions of tubercle bacilli exposed for a long time to lauric acid or oleic acid in concentrations which inhibit growth of bacilli were almost the same in number as those in the control suspension exposed to phosphate buffer.

This fact shows that lauric or oleic acid is not bactericidal but bacteriostatic against tubercle bacilli.

5) Lauric acid or oleic acid in concentrations which do not permit growth of tubercle bacilli (10^{-3}M–10^{-5}M) proved, however, to be stimulative for the oxygen uptake of the bacilli.

6) Oleic acid in relatively high concentrations gave very complicated effects on the metabolism and dehydrogenase activity of the bacilli, showing stimulative effects for certain enzyme systems and inhibitory ones for the other.

Studies on the Immunogenicity of Non-living Tubercle Bacilli with Special Reference to the Relationship between the Immunogenicity and the Activity for Oxygen Uptake in Tubercle Bacilli Killed by Ultraviolet Ray Radiation

Kazuo TAKAHASHI

1) When tubercle bacilli were irradiated by ultraviolet ray beyond certain limit, they were deprived of their activities for growth and for oxygen uptake together. However, if the radiation time was not so long, there existed a stage in which the activity for oxygen uptake was yet retained, though the activity for growth had been lost. This phenomenon was obvious in human and bovine strains of tubercle bacilli, but was not so in avian strains or M. phlei.

2) Human and avian type tubercle bacilli lost their activities for both growth and oxygen uptake gradually with the frequency of repeated freezing and thawing (1–20 times), but M. phlei was indifferent to the same procedure, their activities having been least influenced. The existence of such a strain as M. phlei resistant against freezing and thawing may suggest that the mechanism of the lethal effect of freezing and thawing is different from that of heat or ultraviolet ray.

3) Avian type tubercle bacilli irradiated by ultraviolet ray showed a marked reduction in activity for lactate oxidation, but showed no change in oxidative activity for other substances related to TCA cycle.

4) Of great interest was the finding that ultraviolet ray-irradiated tubercle bacilli which were deprived of growth activity but still retained its activity for oxygen uptake could protect mice against tuberculous infection almost in the same degree as did living BCG bacilli, while the same bacilli which lost its activity for oxygen uptake could no longer protect mice. However, this protective ability varied with strains. For instance, ultraviolet ray-irradiated BCG lacked this ability, though its activity for oxygen uptake was maintained.

The results mentioned above would give some explanations for the discordance of the results so far reported regarding the protective effect of non-living tuberculous vaccines.

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

— Cases Without Transfusion —

Hiroshi NISHIMURA

With the intent of determining the feasibility of non-transfusion in lung resection, the author selected 12 male tuberculous patients. Studies were centered on the measurement of the urine quotient (O/K_4) and measurements were made on the various biological substances in urine and blood.

Condition of blood infusion: Bank blood was infused during and after operation approximately at the same volume as that lost at bleeding.

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

2 day prior to op.	34.6±3.55	4 th day after op.	49.0±4.00
1 day prior to op.	33.2±2.05	5 th day after op.	44.8±3.90
Immediately after op. (0 day)	194 ±55.1	7 th day after op.	36.4±3.01
1 st day after op.	102 ±36.3	9 th day after op.	36.0±1.80
2 nd day after op.	94.4±40.8	12 th day after op.	39.6±3.80
3 rd day after op.	50.8±3.38		

I) The 12 subjects were divided into high and low urine quotient value groups based on the values at 0 days.

The mean values of both groups were 405±59.8 and 60±14.2 respectively.

a) Pre-operatively: It was noted that the subjects in the high group were older (36±3.5), showing lower values in body weight ratio (32.5±0.65) and low values in serum protein (7.34±0.195 g/d*l*), serum potassium (4.1±0.25 mEq/*l*) and serum Cl (104±1.4 mEq/*l*).

b) 0 days after operation: In the high group low values in urine volume (17±4.1 cc/hr) and urinary O/N (1.11±0.169) were seen.

II) At 2-4 days after operation: The subjects were redivided into a high and a low groups. The mean values of both groups were 81.9±26.2 and 46.8±3.6 respectively.

a) During and 0 days after operation: The high group, as compared with the low, showed high values in loss of blood (1400–2000 cc).

b) At 2-4 days after: The high group showed low values in urine Na/K (1.17±0.190), serum Na/K (25.4±1.30) and serum protein (6.98±0.088 g/d*l*).

III) At 9–12 days after operation: The subjects were again divided into a high and a low groups. The mean values of both groups were 46.4±2.3 and 31.9±1.4 respectively.

a) During and 0 days after operation: The high group showed higher values in loss of blood (1500–2200 cc) then the low group.

b) At 9–12 days after operation: The high group showed low values in urine Na/K (1.5±0.34), serum protein (6.98±0.120 g/d*l*) and haematocrit (37±1.5), while showing high values in urine O/N (1.01±0.081).

IV) A comparison was made with Gocho's studies in which supplementary fluid was used. It was noted that low values in serum protein recovered more rapidly in Gocho's results.

The following conclusions were drawn on the assumption that the O/K_4 method may serve as criterion in measuring human vitality.

1. When supplementary fluid is not used, extreme dehydration is seen in the old aged and undernourished patients.

2. When supplementary fluid is not used, abnormal biological reactions (especially a decrease in serum protein) are brought about by loss of blood in large volumes during and immediately after operation.

Studies on the Urine Quotients (O/K_3, O/K_4), as Human Vitality Criteria

— With special reference to cancer patients —

Shohei SEKIGUCHI

Studies were made on cancer patients especially on pre- and post-operative patients in order to determine the limit of the urine quotient method in measuring fatigue.

The experiments led to the following conclusions:

1) In advanced cancer patients (high age level patients with marked metastasis) a remarkable rise was seen in the urine quotient O/K_3, especially in O/K_4, rising to as much as 15 times the normal value (healthy male).

2) Immediately after operation in advanced cancer patients an especially remarkable O/K_4 value which rose to 20 times the normal value was seen. This is the highest value so far encountered by various workers.

Thus, assuming that the patient in question is in a shock state, it may be said that the present O/K_4 method has a wide fatigue measurement range and it may be concluded that it is the only fatigue measurement method in present day medicine.

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

4. Changes in the quantity of the spleen cell constituents in tuberculin cytolysis as observed in tuberculous rabbits

Mikio ITO

Using the suspension culture technic, percentile changes in the quantity of each constituent of the splenic cells of normal and tuberculous rabbits were measured.

During the first week of culture, a conspicuous increase in number of fibrocytes and a decrease in number of lymphocytes and histio-monocytic cells were observed. Addition of tuberculin to the culture media of the splenic cells obtained from tuberculous rabbits caused to stimulate the proliferation of histio-monocytic cells, while fibrocytic proliferation was markedly inhibited in parallel with a decrease in number of lymphocytes and polymorphonuclears.

Immuno-Pathological Studies on the Influence of Prednisolone Combined with Antituberculous Drugs on Experimental Tuberculosis

Hidenoshin KOSUGI

In order to study the mode of action of adrenocortical hormon on the pathogenesis of tuberculosis, tuberculous rabbits and guinea-pigs were treated with prednisolone alone or in combination with antituberculous drugs.

The tuberculin skin reaction and the serum precipitin reaction were tested at the ten day intervals. The contents of the serum protein fractions were measured using paper electrophoresis. The animals were sacrificed group by group at 7 or 10 day intervals. Histological study of the lesions in their viscera was performed.

A remarkable inhibition of the tuberculin reaction occurred following prednisolone injections. The precipitin titer and the γ-globulin content was low in the sera of the animals treated with prednisolone.

Viable bacilli in the spleen and the lungs were less in number in animals given the combined injection than in those treated with antituberculous drugs alone.

Histologically, prednisolone exhibited a marked inhibition against the growth of productive changes and the development of exudative changes.

From the data, it was thought that prednisolone might promote the effect of antituberculous drugs.