

ABSTRACTS

1. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED
ERYTHROCYTES

PART 17. AGGLUTINATION OF ERYTHROCYTES FROM PATIENTS WITH
PULMONARY TUBERCULOSIS BY ANTISERA RESPONSIBLE FOR
MIDDLEBROOK-DUBOS REACTION

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Erythrocytes from 24 out of 67 patients with active pulmonary tuberculosis tested were found to be agglutinated by antisera responsible for Middlebrook-Dubos reaction.

The erythrocytes from 12 out of the 24 patients were pooled and injected intravenously to rabbits, and antibodies capable of agglutinating the OT-sensitized erythrocytes were found to be produced in the animals.

2. STUDIES ON THE INFLUENCE OF NITROUS ACID UPON THE
IMMUNIZING PROPERTIES OF TUBERCLE BACILLI

PART 3. EFFECT OF AGE OF THE BACILLI ON THE
VACCINATING ACTIVITY OF NITROUS
ACID-KILLED TUBERCLE BACILLI

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The present paper describes the results of comparative study on the immunizing activity against tuberculous infection in guinea pigs of four lots of nitrous acid (NA)-killed tubercle bacilli, which differed from one another in age of culture, using living BCG vaccine, as control.

The strain used was virulent human tubercle bacillus, H₂. Four lots of cultures of H₂, grown on Sauton's medium for one, two, four and eight weeks, respectively, were harvested on filter paper. An aliquot of the semi-moist bacilli of each lot was treated with NaNO₂ in acetate buffered solution (pH 4.1-4.2) at 0°C for 8 hours in the same way as was described previously. The NA-killed tubercle bacilli thus obtained were suspended in saline to contain 1 mg of the bacilli per ml (NA-killed H₂ vaccine). The BCG vaccine was prepared from a seven-day-old culture of BCG by grinding the weighed bacilli in a sterile mortar and suspending them in saline to a concentration of 1 mg per ml.

Six groups of guinea pigs weighing from 450 to 500 gm, each consisting of seven animals, were vaccinated intraperitoneally, with a single dose of 1 ml, as follows: Four groups received the NA-killed vaccines, each a different lot, the fifth received BCG vaccine, and the sixth no vaccine. Eight weeks after the vaccination all the animals were challenged with 0.1 mg of virulent H₂. Three months after challenge the 31 animals surviving were sacrificed, and autopsied, and the extent of gross tuberculosis in the viscera was examined. The average Feldman indices for the respective groups were as follows: 1-week-old NA-killed H₂ vaccine, 34.6; 2-week-old NA-killed H₂ vaccine, 42.1; 4-week-old NA-killed H₂ vaccine, 60.9; 8-week-old NA-killed H₂ vaccine, 65.3; BCG vaccine, 52.3; nonvaccinated control, 78.7. From these figures it appears that the NA-killed H₂ vaccines from young cultures are more effective than those from old cultures in stimulating the animal's resistance to subsequent infection with living virulent bacilli.

3. STUDIES ON THE INFLUENCE OF NITROUS ACID UPON THE IMMUNIZING PROPERTIES OF TUBERCLE BACILLI

PART 4. RELATION OF STRAIN TO THE VACCINATING ACTIVITY OF NITROUS ACID-KILLED TUBERCLE BACILLI

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Seven lots of nitrous acid (NA)-killed vaccines prepared from seven different strains of tubercle bacilli, including human and bovine types, were tested for their immunizing activity against tuberculous infection of guinea pigs, using living BCG vaccine as control.

The strains used were (a) human tubercle bacilli; H₂, H37, H37Rv, H37Ra, Aoyama B and Frankfurt, and (b) a bovine tubercle bacillus, No.10. The bacilli harvested from 3- or 4-week-old cultures of each strain on Sauton's medium were treated with nitrous acid for 8 hours in the same way as described in the previous papers. The NA-killed bacilli thus prepared were, after thorough washing, suspended in saline to a concentration of 1 mg (semi-moist weight) per ml. The living BCG vaccine was prepared from 9-day-old cultures.

Male guinea pigs weighing 300 to 360 gm were divided into nine groups of seven animals each. One of the seven groups received, intraperitoneally in a single dose of 1 mg, one of the NA-killed vaccines. The eighth group received 1 mg of BCG vaccine, and the ninth received no vaccine (nonvaccinated control). One month later all the animals were challenged subcutaneously with 0.1 mg of virulent H₂. Two months after challenge all the surviving animals were sacrificed and autopsied, and the gross extent of tuberculous infection in the viscera was examined according to Feldman's scheme.

The results of these immunization tests showed that the NA-killed vaccines from H₂ strain was the most effective of all in inducing resistance of animals to subsequent infection with virulent tubercle bacilli, the effect being somewhat superior to that of BCG, while the vaccines from other strains were all found to be much less potent than BCG vaccine.

4. STATISTIC OBSERVATION ON ACQUISITION OF DRUG RESISTANCE BY TUBERCLE BACILLI IN THE STANDARD CHEMOTHERAPY PRESCRIBED BY THE TUBERCULOSIS PREVENTION LAW

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The drug-sensitivity of the tubercle bacilli from 217 patients with pulmonary tuberculosis who had received standard chemotherapy was examined, and 120, 103 and 95 of them were observed to have SM-, PAS- and INAH-resistant bacilli respectively.

Moreover, drug-resistant bacilli were isolated even from some patients who had never received chemotherapy, though the percentage was low. These data indicated the importance of examining the drug-sensitivity of the bacilli prior to and occasionally during chemotherapy.

5. ELECTRON MICROSCOPICAL STUDIES ON EXPERIMENTAL LUNG EDEMA

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Electron microscopical study was carried out on experimental lung edema of the dog.

In the lung edema produced by Jordan's standard method, there were formation of air bubbles in the cytoplasm of the alveolar epithelium and engorgement of the red blood cells in the capillaries and inter-cellular substance.

In the lung edema produced by destruction of the preoptic areas, similar findings were observed and in addition, desquamation of the alveolar epithelium was noted. Red blood cells were present in the capillaries and intercellular substance, but not to a conspicuous extent.

6. ON THE RELATIONSHIP BETWEEN SERUM PROTEIN AND COLLOIDAL OSMOTIC PRESSURE IN EXPERIMENTAL PULMONARY EDEMA

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The authors studied the changes of the serum protein content and the colloidal osmotic pressure of plasma in the cases of experimental acute pulmonary edema.

The results obtained were as follows :

1) When the various causative factors of pulmonary edema were given to animals, the serum protein content was diminished and the colloidal osmotic pressure of plasma was decreased, but no significant changes were recognized in the serum protein fractions.

2) When the pulmonary edema developed, the changes of the serum protein content and the colloidal osmotic pressure in plasma appeared as a result of the vital reaction due to the pulmonary edema, and those changes would make the further promotion of the pulmonary edema.

7. CLINICAL STUDIES ON ACUTE PULMONARY EDEMA FOLLOWING THORACIC SURGERY

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The author has made observations on 16 clinical cases of postoperative acute pulmonary edema after thoracic surgery.

Among the etiologic factors, hypoxia, disturbance of pulmonary circulation, decrease of pulmonary vascular bed, rapid transfusion and hilus stimulation play major roles in the production of pulmonary edema.

It is necessary for the treatment of the postoperative pulmonary edema to analyse the major causes of the edema properly. In the first step the airway should be opened and sufficient oxygen given. According to the clinical manifestations venesection, postural alteration and intermittent positive pressure respiration should be carried out in combination with administration of morphine, cardiac and diuretic drugs, antihistamine defoaming agents, parasympathoblockings, and adrenal cortical hormones.

8. RESULTS OF ANTI-TUBERCULOSIS MEASURE FOR
TEACHERS IN ISHIKAWA PREFECTURE

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In 1953, out of the 7,331 school teachers in Ishikawa Prefecture examined for health 216 were found to be suffering pulmonary tuberculosis, but in 1959, only 39 out of the 7,474 examined were found to be with the disease.

This remarkable decrease of the number of patients may be attributed firstly to the careful screening of appointees, secondly to the periodical examination of all the teachers, and thirdly to long-term hospitalization and radical treatment of the patients discovered.

9. THE RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURE
OF GELATIN AND ITS ACTIVITY TO RESTORE THE
STREPTOLYSIN-S SUSCEPTIBILITY OF
TANNED ERYTHROCYTES

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Chemical studies were made to obtain information concerning active groups in gelatin molecule responsible for its activity to restore the streptolysin-S susceptibility of tanned erythrocytes.

Erythrocytes of guinea pigs were used throughout. The streptolysin-S employed was a purified sample prepared from 1% ribonucleate-broth cultures of hemolytic streptococci. The tanned erythrocytes were prepared by treating 0.4 ml of 50% suspension of washed red cells with 4 ml of a 1:10,000 solution (in phosphate-buffered saline of pH 7.3) of tannic acid in an ice-water bath for 10 minutes, and by washing with cold buffered saline. Susceptibility of the erythrocytes to streptolysin-S was assayed by hemolytic titration.

The results obtained were as follows :

1) None of the constituent amino acids of gelatin restored the reduced streptolysin-S susceptibility of the tanned erythrocytes.

2) The restoring activity of gelatin was found to be readily destroyed by the crystalline proteinase of *Bac. subtilis*.

3) Neither nitrite nor formalin exhibited any deleterious effect on the biological activity of gelatin.

4) Sulfonation of gelatin with concentrated sulfuric acid at low temperatures resulted in marked decrease of its restoring activity.

10. FURTHER STUDIES ON THE RESTORATION ACTIVITY
OF GELATIN UPON THE STREPTOLYSIN-S
SUSCEPTIBILITY OF TANNED ERYTHROCYTES

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Previous papers of this laboratory reported that erythrocytes of some mammals, when treated with tannic acid, were rendered highly resistant to streptolysin-S, and that various proteins were capable of restoring the lost susceptibility of tanned erythrocytes, gelatin being the most potent. Gelatin is not a homogeneous protein, and therefore, samples of gelatin show considerable differences in chemical and physical properties according to the sources and the methods of preparation.

In the present study seven different samples of gelatin, including highly purified and relatively crude ones, were tested for their activity to restore the streptolysin-S susceptibility of tannic acid-treated erythrocytes of the guinea pig.

0.4 ml of 50% suspension of washed red cells was treated with 4 ml of 1:10,000 solution of tannic acid in the cold for 10 minutes. Then the cells were washed and resuspended in 4 ml of a solution of gelatin to be tested, and incubated at 37°C for 20 minutes. At the end of incubation the cells were washed and resuspended in 20 ml of saline. Finally, the red cells thus obtained were tested for their susceptibility to streptolysin-S by hemolysis test. As control tanned erythrocytes were incubated in saline without added gelatin.

Hemolysis tests showed that all the samples of gelatin tested were equally potent in restoring the reduced susceptibility of tanned erythrocytes to streptolysin-S; thus, 1:20,000 solution of these gelatin samples caused complete restoration of the lysin susceptibility of tanned red cells, which had only one-fortieth of the sensitivity of normal cells, and these samples exerted recognizable effect in dilutions as high as 1:200,000.