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## Abstracts of "Tuberculosis Research" Vol. 7, 1957.

### Hemagglutination Reaction by the Phosphatides of the Tubercle Bacillus

Y. TAKAHASHI and K. ONO

It was shown experimentally that the phosphatides of the tubercle bacillus possess a hemagglutinating activity just as high as or higher than that of the tuberculin polysaccharide. It was also shown by the cross inhibition test that the antigen-antibody system participating in the phosphatide hemagglutination is independent at least of that participating in the tuberculin-polysaccharide hemagglutination.

The samples of phosphatides were obtained from strains of the human tubercle bacillus and of BCG, either according to Boquet and Negre's method, or according to Anderson's method. Sheep erythrocytes were used. The tuberculous sera were those obtained from rabbits immunized with the following bacterial materials: 1) human type whole bacilli killed by immersion in acetone (BA), 2) BA completely exhausted by successive treatment with acetone, methanol and chloroform (RC), 3) RC completely deprived of bound lipids by treatment with 1 per cent hydrochloric acid alcohol. 4) living human tubercle bacilli of low virulence, 5) heat-killed human tubercle bacilli of low virulence and 6) BCG bacilli.

Erythrocytes sensitized with each of the phosphatide samples agglutinated in the presence of anti-sera 1), 4), 5) and 6). They did not agglutinate in the presence of anti-sera 2) and 3), while erythrocytes sensitized with a sample of tuberculin polysaccharide did so in the presence of serum 2). Neither phosphatide nor polysaccharide hemagglutination did occur in the presence of serum 3).

The phosphatide hemagglutination was not in the least inhibited by the tuberculin polysaccharide and vice versa.

### Influence of Egg-Yolk on the Virulence of Mycobacterium Tuberculosis (IV) —With special reference to egg-yolk lipovittelin

Kenichi YAMAMOTO, Schigetoshi TAKAGI, Akio HAGIWARA  
and Isao SEKIKAWA

It was shown in a previous paper that egg-yolk intensifies tuberculin allergy and enhances tuberculous lesions in guinea pigs. Assuming that this enhancing action of egg-yolk on the process of experimental tuberculosis might be in its protein fraction, lipovittelin was isolated from egg-yolk and studied of its behaviour on experimental tuberculosis.

Lipovittelin was prepared according to Chargaff. In parallel with the lipovittelin, an ether fraction which was obtained in the course of lipovittelin preparation was also studied. In a first experiment, guinea pigs were inoculated intraperitoneally with 0.001 mg of virulent bacilli, strain Nakano, suspended in aliquots of distilled water containing 25mg of lipovittelin. An equal number of control animals received the same dose of bacilli suspended in distilled water without lipovittelin. In a second experiment, five groups of guinea pigs were inoculated intraperitoneally with one single injection of 0.001 mg of virulent bacilli, strain Nakano, suspended in the following solutions:

distilled water for group I, 50% egg-yolk solution for group II, aliquots of distilled water containing 50 mg of lipovittelin for group III, aliquots of distilled water containing 25 mg of the ether fraction for group IV, and aliquots of distilled water with 50 mg of lipovittelin plus 25 mg of the ether fraction for the remainder group. After inoculation all the animals were observed with the same method as described in the previous paper.

The experiment led to the following results.

Neither lipovittelin nor the ether fraction of egg-yolk did so exalt tuberculin allergy as egg-yolk itself did, whether they were given separately or concomitantly mixed with virulent tubercle bacilli. Furthermore, no evidence was found that they enhanced tuberculous lesions in the visceral organs and the lymph nodes.

### Studies on the Mixed Cultivation of Acid-Fast Bacilli and *Candida Albicans* (II)

Toshio YOKOI

*Candida albicans* (c. alb.) was cultured on modified Kirchner media mixed respectively with mammalian type, avian type tubercle bacilli, and non-pathogenic acid-fast bacilli for the purpose of observing cultural changes in the mixed organisms following different metabolic phases of the acid-fast bacilli used.

C. alb. was also cultured respectively in culture filtrates of the acid-fast bacilli described above. The filtrates were obtained at various stages of cultivation.

Oxygen consumption of c. alb. and of the acid-fast bacilli used was measured respectively in the presence of the different culture filtrates.

Results obtained were as follows:

1) The multiplication of c. alb. cultured with mammalian tubercle bacilli was markedly promoted, while the growth of the tubercle bacilli was more or less inhibited.

On the contrary, the multiplication of c. alb. inoculated with avian tubercle bacilli or *M. phlei* was suppressed to some extent, while the growth of the latter two was quite prosperous.

But the suppression of the multiplication of c. alb. in this experiment was not so complete as that reported in a previous work in which Sauton media were used.

The incomplete suppression as observed in this experiment is considered to be attributable to the use of modified Kirchner media in which c. alb. is prevented to some extent from being cultured at alkaline pH.

2) Young culture filtrates of acid-fast bacilli promoted the multiplication of c. alb. regardless of strains of acid-fast bacilli used, but older culture filtrates of the bacilli except strain Nakano did not.

3) A parallel relationship was noticed between the degrees of promoting activity of culture filtrates of various acid-fast bacilli upon the multiplication of c. alb. and the oxygen consumption of c. alb. in the presence of culture filtrates of the respective acid-fast bacilli.

4) The growth promoting substance for c. alb. present in tuberculin is thermostable and is of low molecular weight.

### Studies on the Mixed Cultivation of Acid-Fast Bacilli and *Candida Albicans* (III)

Toshio YOKOI

1) When human tubercle bacilli was cultured on modified Kirchner media mixed with c. alb.,

the growth of the bacilli was inhibited to some extent, and when stained, involution forms appeared towards the later stage of cultivation.

At this time, the viable unit of the bacilli decreased remarkably as compared with control cultures without *c. alb.*

Within the first 2 weeks of cultivation, the growth of tubercle bacilli was hardly inhibited in the presence of *c. alb.*

2) When tubercle bacilli were cultured on the filtrate of 2 week mixed cultures, the bacilli formed only thin membranes on the surface of the filtrate, the yields being less than half the control cultures. The mixed culture filtrate seemed thus to be unsuitable for the growth of tubercle bacilli. But, whether this is due to the lack of the energy sources in the filtrate following the multiplication of *c. alb.* is not yet clear.

3) Regardless of strains, both neutral, aqueous extracts and alkali extracts of acid-fast bacilli promoted the multiplication of *c. alb.*

However, the substances in these extract responsible for this promoting effect seemed not to be identical to the growth-promoting substances present in the culture filtrates of human tubercle bacilli and BCG.

4) Culture filtrates of human tubercle bacilli and BCG obtained at any stages of culture promoted the multiplication of *c. alb.*

The older the culture filtrate, the more active its growth promoting effect. On the contrary, in the case of avian type tubercle bacilli and non-pathogenic acid-fast bacilli, the growth promoting effect for *c. alb.* appeared greater in the younger culture filtrates than in the older ones.

### On the Fluctuation in Virulence of the Stock Strain H37Rv as Observed by Repeated Animal Passages

Toshio YOKOI, Goro HIRANO, Shiro ISHIYAMA  
and Eiichiro SUMIKAWA

The stock strain H37Rv subcultured for about 6 years on artificial media were examined on its fluctuation in virulence by repeated passages into guinea pigs or mice. No difference was observed in virulence between the stock strain and the strain which underwent repeated animal passages for about one and half years.

#### Studies on Experimental Tuberculosis in Mice

##### I. Virulence of *M. tuberculosis*, var. *bovinus*, strain Ravenel as observed from survival time of mice.

Takao SHIDA, Goro HIRANO and Shiro ISHIYAMA

Survival times of non-inbred white mice inoculated intravenously with bovine tubercle bacilli, strain Ravenel, were observed.

The results obtained were as follows.

1) Mice weighing 13 g to 20 g injected with 1 mg of the bacilli exhibited the most uniform biological response and all the animals inoculated died within 5 weeks after infection.

From the results obtained, the infecting dose of 1 mg seemed to be most suitable, and ET 50 thereby (50 per cent mortality time in the number of mice) was about 20 days.

2) So long as the suitable dose of the organisms is used for challenge, use of inbred mice is not of special necessity.

In this special case of the strain Ravenel, uniform and reproducible results can be expected

with non-inbred mice.

3) The increase in virulence of the bacilli by the passage through mice was only slight by the second passage.

But the uniformity in mortality of mice increased as the passage of the bacilli was repeated, and the reliability of the experimental results became higher.

4) The degree of weight-loss of mice inoculated seemed not to be always related to the death time of the animals.

5) For calculating 50 per cent mortality time in experiments on chemotherapy and immunity, use of logarithmic probability papers seems to be superior to that of arithmetic probability papers.

### Studies on Antituberculous Immunity Caused by Heat-Killed Tubercle Bacilli in Mice.

Takao SHIDA, Masuo ITAKURA, Tatsuo NUMATA  
and Goro HIRANO

1) Following vaccination with BCG, mice acquired resistance against challenge of virulent tubercle bacilli.

When vaccinated with heat-killed tubercle bacilli incorporated in paraffine oil, mice exhibited an antituberculous resistance to some extent.

This resistance increased even to the level of that induced by vaccination with BCG, when the same dose of vaccination was divided into two doses and injected into the animals at a certain interval of time.

2) Use of mice for experiments on antituberculous immunity proved to be possible.

In experimental tuberculosis in mice, determination of survival time of the animals may serve as a simple and objective criterion for assessment of acquired resistance to tuberculosis.

### Tuberculosis and Urine Quotient

4. Urine quotient ( $O/K_3$ ) as a new criterion for measuring the general ability of human bodies under various conditions and its application to thoracic surgery (I).

Tokuji NOZAKI

A critical study on the above method  $O/K_3$  was carried out on 8 post-operative chest disease patients.

A grand total of 101 measurements of  $O/K_3$  values in the 24 hour urine tests were made on 8 patients; 21 before, and 80 after operation.

1)  $O/K_3$  value of the tuberculous patients prior to operation stood at  $37.1 \pm 7.54$  ranging between 21.3 and 78.9, which showed statistically a higher value as compared to the normal value  $21.9 \pm 4.1$ , and daily  $O/K_3$  values measured in post-operative tuberculous patients under Ringer's solution treatment during the first 3 post-surgical days. The results were as follows.

1st day after operation	$111.6 \pm 44.36$
2nd day	$103.8 \pm 45.39$
3rd day	$84.8 \pm 50.20$
4th day	$45.9 \pm 38.44$
5th day	$40.3 \pm 9.09$

6 th day	35.9 ± 10.05
7 th day	34.1 ± 14.66
9 th day	43.0 ± 24.66
11 th day	43.4 ± 27.67
13 th day	43.7 ± 18.75

As seen above, on the first and second days after operation which is considered to be a period of shock, the O/K<sub>3</sub> values were at a remarkable high level, and in all individual figures a difference was noted statistically against normal values.

In this case, the vakat-O (O) values (the numerator of the quotient; mg per hour) were as follows:

prior to operation	315.2 ± 30.29
1 st- 2 nd day after operation	442.1 ± 117.78
3 rd- 4 th day	446.4 ± 53.46
5 th- 7 th day	403.2 ± 116.96
9 th-11 th day	349.0 ± 69.61
13 th-15 th day	343.3 ± 86.95

On the 1st to 4th day after operation the vakat-O (O) values were at a statistically high level in comparison with those on the other days and on the 1st to 7th day after operation the vakat-O (O) values stood statistically at a high level as compared with the normal value (346.8 ± 59.51).

On the other hand, the K<sub>3</sub> values (the denominator of the quotient, mg per hour) were as follows:

prior to operation	8.80 ± 1.948
1st to 2nd day after operation	5.19 ± 2.292
3rd to 4th day	10.1 ± 5.18
5th to 7th day	12.1 ± 4.90
9th to 11th day	10.0 ± 3.66

As seen above, the K<sub>3</sub> values prior to operation were statistically at a low level as compared to the normal value (16.0 ± 2.56) and the K<sub>3</sub> values on the 1st to 2nd day after operation were statistically at a low level as compared to those on the other days.

2) The values in urine excretion, urinary chloride, urinary nitrogen and O/N measured prior to and after operation in the same patients were as follows:

a) Urine excretion:

1 st to 2 nd day after operation	25.4 ± 7.89
3 rd to 4 th day	39.8 ± 12.98
5 th to 7 th day	40.0 ± 14.30
9 th to 11 th day	40.9 ± 13.35
13 th to 15 th day	55.1 ± 15.73

b) Urinary chlorine (mg per hour):

prior to operation	347.0 ± 50.62
1 st to 2 nd day after operation	157.9 ± 70.50
3 rd to 4 th day	172.6 ± 60.43
5 th to 7 th day	217.0 ± 78.11
9 th to 11 th day	241.2 ± 42.88
13 th to 15 th day	317.8 ± 83.20

c) Urinary chlorine (mg per 100 cc):

prior to operation	830.6 ± 117.0
1 st day after	650.2 ± 223.3
2 nd day	527.3 ± 146.1
3 rd day	434.9 ± 276.9
4 th day	429.6 ± 108.5

5 th day	524.6 ± 245.0
7 th day	563.2 ± 200.0
9 th day	632.6 ± 188.2
11 th day	644.0 ± 197.5
13 th day	615.3 ± 214.5
d) Urinary nitrogen (mg per hour):	
prior to operation	414.6 ± 44.1
1 st to 2 nd day after operation	379.0 ± 135.7
3 rd to 4 th day	536.2 ± 200.0
e) O/N:	
prior to operation	0.81 ± 0.08
1 st day after operation	1.61 ± 0.39
2 nd day	1.19 ± 0.35
3 rd day	0.95 ± 0.36
4 th day	0.97 ± 0.46
5 th day	1.00 ± 0.45
6 th day	1.02 ± 0.33
7 th day	1.02 ± 0.43
9 th day	0.93 ± 0.30
11 th day	1.03 ± 0.41
13 th day	0.91 ± 0.24

3) To observe the relation of the  $O/K_3$  values against the values of the other urinary substances such as urinary nitrogen and chlorine etc., the above eight tuberculous patients were divided into two groups: one shows a high level and the other shows a relatively low level in  $O/K_3$  after operations.

In the former a remarkable decrease in urinary excretion and urinary chlorine after operation was noted and in the latter a low level in urinary nitrogen was observed as compared with levels during the period of observation.

### Stress and Urine Quotient

1. Urine Quotient ( $O/K$ ,  $O/K_2$ ) as criteria for measuring the general ability of human bodies under various conditions and their application to a study on alcohol stress.

#### Yūji NAKAYAMA

Clinical and laboratory studies were carried out on 12 normal adults in order to observe the metabolic consequences produced by alcohol agents.

The above men, prior to the administration of alcohol (200 cc of 25% alcohol) were divided into the following 3 groups: A-control, B-previously subjected to 4 hours work and C previously subjected to 8 hours work.

Determinations on urine included urine quotients ( $O/K$ ,  $O/K_2$ ), urine excretion, phosphorus, chlorine, Donaggio value (D.V.) and Vakato/nitrogen ( $O/N$ ) together with blood including erythrocyte sedimentation rate (S.R.) were made prior to the administration of alcohol per os every hour and a 1/2 for 4 hours and 1/2 (duration of experiment).

1) The values of urine quotients increased following the administration of alcohol, in C group, while the values of  $K$  and  $K_2$  (the denominators of the urine quotients: mg per hour) decreased, especially in C group.

2) Urinary Vakato-O ( $O$ ), Nitrogen and  $O/N$  were also increased without any significant difference

in the above three groups.

3) D.V. showed an insignificant change during the course of experiments, while the S.R. showed a definite increase 1 hour after the administration of alcohol, without any significant difference in the three groups.

4) The administration of alcohol resulted in increases of urine excretion (cc per hour) and concentration (mg per 100 cc), especially in C group.

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

### 1. The Role of Complement in Cytolysis by the Specific Antigen

Mikio ITO

Using the suspension culture technic, the action of the specific antigen against splenic cells of sensitized rabbits was studied with special reference to the role of complement in culture media.

Old tuberculin and egg albumin, which had no effect on the proliferation of normal cells, showed a remarkable inhibitory effect on that of cells of rabbits previously injected with BCG or egg albumin, respectively.

When sera heated at 56° C for 30 minutes were used as a constituent of culture liquid media, the inhibition was not observed.

From this it is evident that complement plays a great role in cytolysis by the specific antigen.

## Experimental Studies on the Biological Properties of Extracted Cells of *Myc. tuberculosis*

### II. Immunization Experiments

Shigetoshi TAKAGI

In order to verify the immunizing capacity of the three kinds of extracted cell residues (BA, RC and RHA) reported in the preceding paper, the following experiments were undertaken.

Rabbits previously injected with the residues together with normal animals as control were challenged with virulent human tubercle bacilli. They were sacrificed at different intervals. The visceral organs were studied histologically and bacteriologically.

A strong positive skin reaction was evoked by tuberculin injection in animals injected with BA. A weak but specific positive tuberculin reaction was observed in those injected with RC or RHA.

Twenty-four hours after challenge severe exudative changes were observed in the lungs and spleen of the animals previously inoculated with the residues. These changes lasted only for a short period of time. The animals showed also a formation of tubercles earlier than control. However, these changes were somewhat weaker in the animals inoculated with RHA. On the 33rd day after challenge tuberculous lesions were far weaker in the viscera of the animals inoculated with the residues, especially with RC or BA, than in those of control.

Viable colony counting presented similar results to the histological findings.

Judging from the data above described, RC and BA can be said to possess a marked immunizing capacity against tuberculous infection. RHA has only a slight, though irrefutable, immunizing capacity.