

# **ABSTRACTS**

## 1. STUDIES ON THE INFLUENCE OF ANTITUBERCULOUS AGENTS UPON TISSUE RESPIRATION

### PART 4. INFLUENCE OF PZA PLUS INAH ON TISSUE RESPIRATION IN EXPERIMENTAL TUBERCULOSIS

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The following 5 groups of mice were subjected to the experiments to investigate the influence of PZA and INAH upon tissue respiration.

Group A. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv"

Group B. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv" and treated with PZA

Group C. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv" and treated with INAH

Group D. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv" and treated with PZA and INAH

Group E. Normal mice

The results obtained are summarized as follows:

1) In splenic and pulmonary tissue respiration, groups A, B, C and D were observed to have no difference among them, but each was found to be above group E. On the other hand, in hepatic tissue respiration, no difference was observed among the five groups.

2) The hepatic tissue respiration of groups C and D was strongly suppressed by INAH added to the medium in a high concentration (1~10 mg/ml), while a low concentration of INAH produced acceleration of the splenic and pulmonary tissue respiration in all the groups except group B, in which no change was observed.

3) The influence of PZA added to the medium in a high concentration upon the splenic and pulmonary tissue respiration was about the same in groups C and D. A low concentration of PZA produced acceleration of the hepatic and pulmonary tissue respiration in group D, which was not observed in group B or C.

4) The observation of tissue respiration was not helpful in understanding why the combination PZA plus INAH should be so much more effective for therapeutic purposes than either drug alone.

## 2. EXPERIMENTAL STUDIES ON THE COMBINED ADMINISTRATION OF ANTITUBERCULOUS DRUGS AND TUBERCULIN

### PART 1. EFFECT OF OT AND O-AMINOPHENOL AZO-TUBERCULIN ON PAS, O-AMINOPHENOL AND SM TREATMENT

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A series of experiments was carried out upon guinea pigs infected with 0.1 mg H<sub>2</sub> strain of human type tubercle bacilli for testing how the effectiveness of PAS, o-aminophenol (OM) and SM is affected when each of the drugs was supplemented with OT or o-aminophenol azo-tuberculin (OA-Azo-T).

The simultaneous use of OA-Azo-T increased the effectiveness of SM and of OM, but not of PAS. In the former the best result was obtained when the dosage of OA-Azo-T was 0.000,05  $\gamma$  every five days.

As to OT, concentration as low as 1 in 1,000,000 produced severe focal lesion but did not enhance the curative power of any of the antituberculous drugs used in the experiments.

### 3. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 19. IMMUNOLOGICAL PROPERTIES OF EXTRACTS  
OF TUBERCLE BACILLI  
No. 1. CORRELATION BETWEEN EXTRACTING CONDITION  
AND ANTIGENICITY OF THE EXTRACTS

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Extracts of human tubercle bacilli "Aoyama B" prepared under various conditions were tested for their power of sensitizing red cells and of causing precipitin reaction and skin reaction.

The results obtained are summarized as follows:

1) High potency was observed in the extract prepared from the bacillary suspension placed in an ice-box for seven days after heating at 100 °C for 1 hour.

2) Aquous and saline extracts were found to be more powerful in sensitizing red cells and causing precipitin reaction than citrate extract, but less powerful in causing skin reaction.

In passing, the substance causing skin reaction was not observed to be adsorbed by red cells.

#### 4. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

PART 19. IMMUNOLOGICAL PROPERTIES OF  
EXTRACTS OF TUBERCLE BACILLI  
NO. 2. ON THE AQUOUS EXTRACT OF  
TUBERCLE BACILLI

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In the preceding study, the aqueous extract of human type tubercle bacilli prepared from the bacillary suspension placed in an ice-box for seven days after heating at 100 °C for 1 hour was observed to have high serological reactivities.

In the present study, the antibody producing ability of the extract and extract-sensitized red cells was examined and compared with that of OT-sensitized red cells and of heat-killed bacilli.

The results obtained were as follows:

1) In the ability of producing circulating antibodies, the extract-sensitized red cells, OT-sensitized red cells and the heat-killed bacilli were almost equal to each other, and superior to the extract.

2) But these four immune sera were deprived of all their serological activities (hemagglutinating and hemolysing activities for the sensitized red cells and precipitating activity against the extract) by absorption with the heat-killed bacilli, but retained most of the precipitating activity against the extract or OT after absorption with the extract-sensitized or OT-sensitized red cells.

#### 5. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 12. SIGNIFICANCE OF THE RETICULOENDOTHELIAL SYSTEM  
IN THE IMMUNIZATION WITH OT-SENSITIZED RED CELLS  
NO. 1. EFFECT OF VARIOUS PRE-TREATMENTS GIVEN TO THE  
RETICULOENDOTHELIAL SYSTEM ON THE PRODUCTION  
OF ANTIBODIES

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The following results were obtained from observation of the effects of india ink injection, splenectomy, X-ray irradiation and internal irradiation with  $\text{Cr}^{51}$  on the production of antibodies by OT-sensitized red cells.

1) Injection of india ink and splenectomy were found to have conspicuous inhibitory action on the production of agglutinin and hemolysin for OT-sensitized red cells. The action was especially strong on the hemolysin production. There was hardly any difference of potency between the two kinds of pre-treatment.

2) X-ray irradiation given 6 hours before the start of the immunization proved to be without effect when the dosage was 50 roentgens, but to be inhibitory when it was 600 roentgens.

3) The highest potency for producing antibodies for OT-sensitized red cells was exhibited by the animal's own red cells sensitized with OT, followed by  $\text{Cr}^{51}$ -labeled, OT-sensitized sheep red cells and the animal's own OT-sensitized red cells labeled with  $\text{Cr}^{51}$ , in that order.

4) Injection of the animal's own  $\text{Cr}^{51}$ -labeled red cells showed only a very slight inhibitory effect on the production of antibodies by subsequent immunization with OT-sensitized red cells.

## 6. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

PART 12. SIGNIFICANCE OF THE RETICULOENDOTHELIAL SYSTEM  
IN THE IMMUNIZATION WITH OT-SENSITIZED RED CELLS  
NO. 2. EFFECT OF VARIOUS PRE-TREATMENTS GIVEN TO THE  
RETICULOENDOTHELIAL SYSTEM ON THE DISTRIBUTION  
OF OT-SENSITIZED RED CELLS IN DIFFERENT ORGANS

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The author investigated the effect of various pretreatments such as blocking the RES with india-ink, splenectomy, X-ray irradiation and internal irradiation with  $\text{Cr}^{51}$ , on the rate of intravascular survival of antigen-sensitized red cells injected intravenously.

The effect was traced in immunized and non-immunized rabbits by the  $\text{Cr}^{51}$ -labeling method. The distribution of  $\text{Cr}^{51}$  in different organs after intravenous injection of  $\text{Cr}^{51}$ -labeled red cells was also measured.

The results obtained were as follows.

1) In non-immunized rabbits, neither india-ink blockade nor splenectomy showed

any noticeable effect on the rate of intravascular survival of the injected OT-sensitized cells.

2) The rate of intravascular survival of the OT-sensitized cells injected after india-ink blockade or splenectomy showed no significant difference whether or not the animal had been immunized with its own red cells sensitized with OT.

3) The rate of intravascular survival of OT-sensitized red cells injected to animals diminished more rapidly in those immunized with OT-sensitized red cells of their own and then splenectomized than in non-immunized ones, but somewhat more slowly than in immunized animals with their spleens intact.

4) X-ray irradiation reduced the life of OT-sensitized red cells in the blood stream when the dosage was 50 r, but prolonged it when the dosage was 600 r.

5) Normal sheep red cells, OT-sensitized sheep red cells and OT-sensitized red cells of the animal's own were seen to survive longer in the circulation when injected to animals previously given five injections of Cr<sup>51</sup>-labeled OT-sensitized sheep red cells than when injected into animals receiving non-labeled OT-sensitized sheep red cells.

6) When Cr<sup>51</sup>-labeled, OT-sensitized red cells of the animal's own were injected to normal rabbits the Cr<sup>51</sup> showed a tendency to accumulate in the liver, spleen and bone marrow.

In the case of Cr<sup>51</sup>-labeled sheep red cells the Cr<sup>51</sup> was quickly excreted through the kidney, while the Cr<sup>51</sup> of labeled red cells of the animal's own gradually accumulated in the spleen and bone marrow.

7) When labeled, OT-sensitized red cells of their own were injected to rabbits immunized with OT-sensitized red cells, the Cr<sup>51</sup> was seen to be quickly excreted through the kidney, but their livers showed higher concentration of Cr<sup>51</sup> than the livers of non-immunized animals.

8) Splenectomy did not seem to exercise any influence on the distribution of Cr<sup>51</sup> in other organs, either in those animals which had been immunized with OT-sensitized red cells or in non-immunized ones, following injection of labeled, OT-sensitized red cells of their own or labeled sheep red cells.

In non-immunized animals, injection of india-ink did not bring about significant difference in the distribution of Cr<sup>51</sup> in organs.

## 7. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

### PART 13. USE OF <sup>51</sup>CR-LABELING METHOD FOR IMMUNOLOGICAL STUDIES

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For the purpose of clarifying the antigenicity of red cells sensitized with OT, its protein fraction (PF) and its polysaccharide fraction (CF),  $^{51}\text{Cr}$ -labeled recipients' own red cells sensitized with OT, PF and CF were injected intravenously to the rabbits of the following two groups and the survival of the labeled red cells in the circulation was observed.

Group I. Normal rabbits

Group II. Rabbits immunized with

- |                  |                             |
|------------------|-----------------------------|
| a) OT-sensitized | } recipients' own red cells |
| b) PF-sensitized |                             |
| c) CF-sensitized |                             |

The results obtained were as follows:

1) The  $^{51}\text{Cr}$ -labeled red cells sensitized with OT, PF or CF disappeared faster from the circulation after the injection in group II than in group I. Thus it is to be inferred that OT and its fractions have the power of sensitizing red cells and the sensitized cells are capable of producing circulating antibodies.

2) The sensitized red cells disappeared from the circulation of the immunized rabbits with or without intravascular hemolysis, according as the animal's serum shows hemolytic activity in vitro toward OT-sensitized red cells or it merely causes hemagglutination.

3) From the data presented it was shown that the  $^{51}\text{Cr}$ -labeling method is usable for the study of antigen-antibody reaction in vivo.

## 8. IMMUNOLOGICAL STUDIES ON OLD-TUBERCULIN-SENSITIZED ERYTHROCYTES

### PART 14. MECHANISMS OF ANTIBODY PRODUCTION

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A series of experiments was carried out, by means of the  $^{51}\text{Cr}$ -labeling method, to study the correlation between the fate of the OT-sensitized red cells in the circulation after the injection and the antibody production.

- 1) When injected intravenously, OT-sensitized red cells showed higher antibody-

producing activity than OT-sensitized trypsinized red cells or the hemolysate of OT-sensitized red cells.

2) Intravenous injection of OT-sensitized red cells was observed to be more effective for antibody production than intraperitoneal or subcutaneous one.

3) From the data presented it was shown that long survival of OT-sensitized red cells in the circulation is a necessary condition for promoting the antibody production.

## 9. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN

### PART 21. DIFFERENCE OF THE REACTION ACCORDING TO THE LOCALITY OF INJECTION

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The difference of tuberculin skin reaction according to the locality of injection was observed upon 3,766 school children. They were given intracutaneous injection of OT, o-aminophenol azo-tuberculin "Human" or o-aminophenol azo-tuberculin "BCG" at two locations of one forearm or at the corresponding locations on both forearms and the resulting reactions were compared. Most of the children had been inoculated with BCG, and the rest, infected naturally with tubercle bacilli. All of them had been undergoing annual examination of tuberculin skin reaction on the inside of the left forearm.

The results obtained are summarized as follows:

The skin reaction observed 48 hours after the injection of any kinds of tuberculin is nearly uniform in all the locations. Therefore, the author believes that, choice of the locality for injection is immaterial for this type of test.

## 10. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN

### PART 22. ON THE ZONE PHENOMENON

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The existence of the zone phenomenon of tuberculin skin reaction was examined

upon 8,022 children and students. They were divided into two groups, one infected with BCG and the other experiencing natural infection. Each of these groups was again divided into three subgroups, and each subgroup was tested for tuberculin skin reaction by injection of OT, o-aminophenol azo-tuberculin "Human" (OA-Azo-T "Human") or o-aminophenol azo-tuberculin "BCG" (OA-Azo-T "BCG").

The summarized results of the experiments are as follows:

1) In every one of the three tuberculins, the standard solution gave a higher degree of reaction, in general, than more dilute solutions.

2) In all subgroups, however, there were some subjects who showed a higher degree of reaction by the dilute solution than by the standard solution.

Namely, the zone phenomenon is present not only in OT but also in OA-Azo-T, and this should be remembered in carrying out tuberculin skin test.

## 11. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN

### PART 23. THE ACTIVITY OF O-AMINOPHENOL AZO-TUBERCULIN "HUMAN" AND O-AMINOPHENOL AZO-TUBERCULIN "BCG" FOR CAUSING SKIN REACTION IN BCG-INOCULATED PERSONS

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It has been confirmed by past researches that, for naturally infected persons, o-aminophenol azo-tuberculin "BCG" (OA-Azo-T "BCG") 0.25  $\gamma$ /0.1 ml is equivalent in activity for causing skin reaction to o-aminophenol azo-tuberculin "Human" (OA-Azo-T "Human") 0.05  $\gamma$ /0.1 ml and for BCG-inoculated persons, the former is more active than the latter.

In the present study, the author compared the activity for causing skin reaction in the intrascapular space between OA-Azo-T "Human" (0.05 $\gamma$ /0.1ml) and OA-Azo-T "BCG" (0.25 $\gamma$ , 0.1 $\gamma$ , 0.05 $\gamma$ /0.1ml) upon 304 school children who have reliable record of BCG inoculation given in the preceding year.

The results obtained were as follows:

In the activity for causing skin reaction, the following relation was observed. OA-Azo-T "BCG" 0.25 $\gamma$ /0.1ml  $\gg$  OA-Azo-T "BCG" 0.1 $\gamma$ /0.1ml  $>$  OA-Azo-T "Human" 0.05 $\gamma$ /0.1ml  $>$  OA-Azo-T "BCG" 0.05 $\gamma$ /0.1ml

These results and the results of the previous researches mentioned before show together that each of the two kinds of tuberculin exhibits different antigenicity toward naturally infected and BCG-inoculated subjects.

## 12. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN

### PART 24. DIFFERENCE OF LOCAL COMPLICATIONS AND TUBERCULIN ALLERGY, DUE TO DIFFERENCE OF DOSAGE, AFTER THE FIRST EXPERIENCE OF BCG

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BCG inoculation was given to two groups of nurslings and infants having no experience of previous inoculation, one group being given 0.05 mg each and the other, 0.1 mg, and the local change produced and the sensitivity to OT and to OA-Azo-T "BCG" conferred were observed.

The results obtained were as follows:

- 1) The local complication was slight in both groups and showed only negligible difference between the groups. There was no formation of ulcer or abscess in either group.
- 2) There was no difference of tuberculin sensitivity between the groups in spite of the difference in quantity of the BCG inoculated.
- 3) OA-Azo-T "BCG" 0.25 $\gamma$ /0.1ml produced stronger reaction than OT 1/2,000, 0.1ml.
- 4) There were some cases which showed strongest tuberculin reaction two years after the BCG inoculation.

Secondly, 3 groups of guinea pigs, (A) inoculated with 5 mg of BCG, (B) inoculated with 0.1 mg of BCG and (C) control, were tested for their local complications and tuberculin sensitivity 4 weeks after the BCG inoculation, then infected with human tubercle bacilli H<sub>37</sub>Rv. Six weeks later, all were autopsied and quantitative culture of the bacilli in viscera was carried out.

The results obtained were as follows:

- 1) Group A showed larger local complication than group B after the BCG inoculation.
- 2) Tuberculin reaction was more remarkable in Group A than in group B.
- 3) Both groups were more sensitive to OA-Azo-T "BCG" than to OA-Azo-T "Human".
- 4) The quantity of the BCG used in the inoculation had no bearing on the defensive power against infection of tubercle bacilli.

## 13. STUDIES ON O-AMINOPHENOL AZO-TUBERCULIN

### PART 25. DIFFERENCE OF TUBERCULIN REACTION EXHIBITED BY DIFFERENT GROUPS, IN PARTICULAR BY BCG-INOCULATED SUBJECTS

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The method of using OT and o-aminophenol azo-tuberculin together for distinguishing natural infection from infection by BCG was re-examined and the following results were obtained.

- 1) As has been repeatedly reported, the method was satisfactory for experimental animals.
- 2) The method gave good results for human subjects in 1949 through 1952, but has recently become unreliable, probably on account of the improvement in potency of BCG vaccine and increased percentage of those subjects getting repeated BCG-inoculation.

#### 14. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 12. STUDIES ON INTESTINAL BACILLI  
 NO. 1. ANTIBACTERIAL EFFECT OF VARIOUS CHEMICALS IN VITRO

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The minimum bacteriostatic and the bacteriocidal concentration of SM, CM, AM, penicillin (Pc), guanofuracin (GF), sulzol (Sul), PAS, INAH and phenol (Ph) to *Salmonella paratyphi B* and *Shigella flexneri 2a* were determined in their cultures in pepton water media.

The results of the experiments were as follows:

- 1) SM showed only a smaller gap between the minimum bacteriostatic and the minimum bacteriocidal concentrations for each of the strains and therefore behaved as a bacteriocide.
- 2) CM, AM, TM and Pc showed large gaps between the minimum bacteriostatic and the minimum bacteriocidal concentrations for each of the strains and therefore behaved as bacteriostatic agents.
- 3) Sul was observed to be bacteriocidal to *Salmonella paratyphi B* but bacteriostatic

to *Shigella flexneri* 2a.

4) PAS and INAH showed scarcely any antibacterial effect to either bacillus.

## 15. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 12. STUDIES ON INTESTINAL BACILLI  
NO. 2. ON THE EFFECT OF SIMULTANEOUS EXPOSURE TO  
TWO CHEMICALS (1)

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The variation of the bacteriostatic action of SM on *Salmonella paratyphi* B and on *Shigella flexneri* 2a in pepton water culture media caused by addition, individually, of AM, TM, penicillin (Pc), guanofuracin (GF), sulzol (Sul), PAS and INAH were studied. Same studies were made also with CM used in place of SM.

The results of the experiments were as follows:

AM, TM, Pc, GF and Sul were effective in enhancing the bacteriostatic power of both SM and CM toward the two microbes, but PAS and INAH showed little enhancing effect with SM and none at all with CM.

## 16. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

PART 12. STUDIES ON INTESTINAL BACILLI  
NO. 2. ON THE EFFECT OF SIMULTANEOUS EXPOSURE  
TO TWO CHEMICALS (2)

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A) The action of AM, TM, penicillin (Pc), guanofuracin (GF), sulzol (Sul), PAS, o-aminophenol (OM) and INAH on the development of resistance of *Salmonella paratyphi* B to SM was studied by means of successive culture of the microbe in pepton water media containing SM and one of the first-named drugs. Same experi-

ments were carried out with CM used in place of SM.

The results of the experiments were as follows:

1) AM and TM showed effects of retarding the appearance of SM-resistant strain.  
2) GF, Sul, PAS and OM showed effects of retarding the appearance of CM-resistant strain.

B) Using the same experimental method, development of SM- and CM-resistance in *Shigella flexneri* 2a was examined.

1) AM, TM, Pc, GF and Sul showed strong effects of retarding the appearance of SM-resistant strain and INAH showed a less strong effect.

2) Pc, PAS and OM showed effects of retarding the appearance of CM-resistant strain.

C) Using the same experimental method, development of AM-, TM-, Pc-, GF- and Sul-resistance of the bacilli was examined.

1) SM showed strong effect for retarding the appearance of TM- and GF- resistance in both bacilli.

2) SM and CM were observed to inhibit completely the appearance of GF- resistant strain of *Salmonella paratyphi* B.

It is of great significance that INAH and PAS which show no antibacterial power by themselves toward either of the two microbes are effective for retarding the appearance of SM- and CM-resistance in the bacilli.

#### 17. ACTIVITY OF OLD TUBERCULINS PREPARED FROM SM-RESISTANT, SM-DEPENDENT AND SM-ENHANCED TUBERCLE BACILLI FOR CAUSING SKIN REACTION

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The potency of OTs from SM-resistant strain, SM-dependent strain and SM-enhanced strain of human tubercle bacillus for causing skin reaction was tested on 62 patients with pulmonary tuberculosis, with the OT from SM-sensitive human tubercle bacilli "H<sub>37</sub>Rv" as a standard of comparison. No statistically significant difference of potency was observed between the four OTs.

#### 18. INFLUENCE OF TUBERCULIN UPON TISSUE RESPIRATION OF TUBERCULOUS MICE

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The following three groups of mice were subjected to the experiment to investigate the influence of OT and o-aminophenol azo-tuberculin (OA-Azo-T) upon tissue respiration.

Group A. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv"

Group B. Mice infected with human tubercle bacilli "H<sub>37</sub>Rv" and treated with PZA

Group C. Normal mice

The results obtained are summarized as follows:

1) By OT in a high concentration (10 mg/ml), i) the hepatic tissue respiration was accelerated most strongly in group C and the degree of acceleration in groups B and A came next in that order, ii) the splenic tissue respiration was suppressed in all the 3 groups, and iii) the pulmonary tissue respiration remained unaffected in all the groups.

On the other hand, a low concentration (0.000,1 mg/ml) of OT suppressed the hepatic respiration of groups A and B, accelerated the splenic of group B and gave no change to the pulmonary in any of the groups.

2) By OA-Azo-T in a high concentration (10  $\gamma$ /ml), the pulmonary tissue respiration in group C was accelerated, while the hepatic and pulmonary ones in group A were suppressed. On the other hand, a low concentration (0.000,1  $\gamma$ /ml) of OA-Azo-T gave no change to the tissue respiration in any group.

## 19. HISTOLOGICAL STUDIES ON TUBERCULIN ALLERGY

PART 4. COMPARATIVE HISTO-CHEMICAL STUDY ON THE INTRACUTANEOUS  
 REACTION PRODUCED BY OLD TUBERCULIN AND o-AMINOPHENOL  
 AZO-TUBERCULIN "HUMAN" AND "BCG"

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The skin test was carried out with OT, OA-Azo-T "Human" and OA-Azo-T "BCG" on the following three groups of rabbits.

A. Rabbits infected with human-type tubercle bacilli, H<sub>37</sub>Rv

B. Rabbits inoculated with BCG

C. Normal rabbits, for control

The three varieties of tuberculin were injected one immediately after another at three spots 5 cm apart in a row on the animal's back. The skin of the parts receiving the injection was removed at stated intervals of time and examined for histological and histo-chemical changes. The followings are a summary of the experiment.

1) The histological examination showed that the reaction produced in group A by OA-Azo-T "Human" was almost the same as that produced by the others, though the initial (within 12 hours) appearance of polymorphonuclear cells was less numerous, and that OA-Azo-T "Human" produced in group B only some cell infiltration and vascular reaction which were much less marked than those produced by either of the other two.

2) The following techniques were employed in the histo-chemical study.

i) For enzymes:

- a) Gomori's alkaline phosphatase staining
- b) Gomori's acidphosphatase staining
- c) Gomori's lipase staining

ii) For polysaccharides:

- a) Lillie's method
- b) Hale's acid staining
- c) Double staining by Hale's method and PAS
- d) Ohno's metachromasia method

iii) For nucleic acids:

- a) Feulgen reaction
- b) Brachet's pyronin methyl green staining

iv) For proteins and amino acids:

- a) Ohno-Kurata-Haga method
- b) Basic amino acid staining

v) Weigert's staining for cellulose

## RESULTS

i) The reaction for alkaline phosphatase found on the infiltrating polymorphonuclear leucocytes was less intense and less permanent when the skin reaction was caused by OA-Azo-T "Human" than when it was by OT.

Also the former brought about less frequently than the latter a positive coloring reaction in the wall of small blood vessels and connective tissue. These differences were more conspicuous in group B than in group A.

ii) The degree of positivity of PAS staining ran roughly parallel to the intensity of tuberculin reaction. OA-Azo-T "Human" produced in the early stage less intense PAS-positive reaction than OT in group A, and throughout the whole course less

intense PAS-positive reaction than either of the other two in group B.

iii) The intensity of the blue color produced by Hale's method reached the maximum in the last stage of the tuberculin reaction.

The intensity seen in animals of group B receiving OA-Azo-T "Human" was less, throughout the whole course, than that seen in animals of the same group receiving either of the other tuberculins.

iv) Only a small number of cells containing pyroninephil substances was observed to appear throughout the whole period of the reaction.

The results shown above prove that it is possible to distinguish rabbits infected with human-type tubercle bacilli from those infected with BCG by the combined use of the two ortho-aminophenol azo-tuberculins, "Human" and "BCG".

## 20. HISTOLOGICAL STUDIES ON TUBERCULIN ALLERGY

### PART 5. HISTOCHEMICAL STUDIES ON ARTHUS PHENOMENON

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Arthus phenomenon produced by egg-albumin in rabbits was observed histologically and histochemically.

The results obtained were as follows:

#### 1) Histological findings:

The cellular infiltration begins with prominent increase of polymorphonuclear leucocytes (3-24 hours after the antigen injection) which is followed by increase of mononuclear cells which, however, do not become as numerous as the polymorphonuclear cells till the third day. The inflammatory changes, such as cellular infiltration, vascular lesions and edematous swelling of the connective tissue, reached their maximum twelve or twenty-four hours after the injection of the antigen, and then decreased gradually.

#### 2) Histochemical findings:

a) The reaction for alkaline phosphatase was found with higher positivity in the infiltrating polymorphonuclear leucocytes than in other cells. Sometimes, positive reaction for alkaline phosphatase was found in small vessels and connective tissue.

b) The positivity of PAS staining ran parallel to the progress of Arthus phenomenon, and was highest 12 or 24 hours after the injection.

c) The positivity of Hale's staining ran parallel to PAS staining.

d) The cells containing pyroninephil substance were observed to appear from

the early stage of the Arthus phenomenon and the number reached a maximum 48 or 72 hours after the injection.

3) From the histological and histochemical point of view, no qualitative difference was observed between Arthus phenomenon and tuberculin reactin.